# Investigations on population structure and intra-specific genetic diversity of Indian Scad, *Decapterus russelli* (Ruppell, 1830) from Indian waters.

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**REPRINTS**

# CHAPTER 1
# GENERAL INTRODUCTION

## 1.1 Introduction

Marine ecosystems provide essential resources and services, with fishes being of prime socio-economic importance (Arthington et al., 2016). Fish are the primary food source for humans among all the aquatic animals and plants. In most civilizations, they are the primary component of the diet and serve as a key source of protein for a large number of people. In addition to providing a substantial amount of the protein that is suitable for human consumption, fisheries and fisheries related activities are profitable industries that create jobs, attract investment, and help many nations balance their trade with other nations.

The majority of fish that is consumed by humans is obtained by exploiting natural populations. There are alarming global trends of excessive exploitation (Lotze 2004) and habitat degradation (Turner et al., 1999) of marine fishes, with most commercial stocks either overfished or nearing capacity (Garcia and Grainger 2005; Hutchings et al., 2010; Srinivasan et al., 2010; Branch et al., 2011). Global climate change is also altering patterns of marine biodiversity (Lam et al., 2020), with more pronounced effects expected in the future. The consequences may lead to irreversible loss of fisheries resources (Turner et al., 1999; Rogers and Beets 2001; Gallastegui et al., 2002), leading to reduced food security and even direct threats to ecosystem integrity. There is a need, therefore, to improve the scientific knowledge base that forms the keystone of sustainable management of fishery resources. In order to manage a fishery effectively, it is important to know the identity of stock structure of the species (Begg and Waldman 1999). Beside this, understanding the genetic mechanisms that determine how marine fishes perform, in relation to environmental conditions, will also contribute to conservation activities by providing insights into current and future species abundance and distribution (Sebastian et al., 2020). In addition, studies on their population structure will also contribute to detect how this underlying genetic and ecological mechanism leads to the differentiation of their population in correlation with various environmental conditions along their geographical range (Coyle 1998).

For the past three decades, mitochondrial DNA (mtDNA) has been the most widely used marker of choice for assessing the molecular diversity in animals, even though it makes up only a very small portion of the genome size of an organism. Although mtDNA is generally considered a good molecular marker for phylogenetic analysis of fish taxa (Yang et al., 2018), short mitochondrial gene fragments have limitations in resolving complicated phylogenetic

relationships in many fish lineages (Kocher and Stepien 1997). The additional informative sites from longer DNA sequences (e.g., mitochondrial genomes) allow for more complete resolution of these deeper branches and higher-level relationships (Miya and Nishida 2000). The organization of fish mitochondrial genomes are similar to other vertebrates (Kartavtsev et al., 2007). The characteristics including constant gene content (Yang et al., 2018), multiple copies per cell (Satoh and Kuroiwa 1991), lack of recombination constraints (Rokas et al., 2003) make the mitochondrial genome an easier system for analysing molecular dynamics and mechanisms of rearrangements that explains variations in the genome than nuclear genome. Additional features such as its compact gene arrangement, increased rate of evolution, uniparental (maternal) inheritance and small size make the mitochondrial genome a very effective tool in the field of phylogenetic and evolutionary biology (Moritz et al., 1987; Wolstenholme 1992).

The sequencing of whole mitogenome will provide novel findings for phylogenetic and evolutionary studies at multiple levels. Because mtDNA variations within a species is thought be connected with the amount of time since a maternal ancestor was last shared (Avise et al., 1986), MtDNA analysis has also been used to date events in a species' history. This time period could be associated with a temporary reduction in the population at some point in the past or the origin of a new species (Neigel and Avise 1985; Wilson et al., 1985). Furthermore, information on codon usage, start and stop codons, and overlapping genes could provide information on molecular evolution. In addition, the secondary structure of the control region and ribosomal genes could also be providing adequate information to delineate the phylogenetic and evolutionary relationship.

An accurate phylogeny is essential to fully understand vertebrate evolution. Therefore, questions of vertebrate relationships have been a primary focus in the field of molecular systematics (Meyer and Zardoya 2003). Fossils can provide evidence of morphological changes (Mongiardino Koch et al., 2021), but the genetic changes associated with evolution can only be recognized through comparisons between extant taxa (Whitehead and Crawford 2006). This is where the need for mitogenome characterization arises, through which one can delineate the relationship among extant taxa and thereby provide accurate results regarding evolutionary relationship at the genetic level.

Although a large number of studies on genetic structure of population have been published in the last decades, data are still rare for most exploited species. The fishes of the family Carangidae are an important group of exploited pelagic resources of India (Murty 1991). Carangid fishes are well suited for developing strategies for sustainable fisheries because of their natural pattern of variation, which is characterized by a 'steady situation' meaning that they do not show large variations in their catches from year to year (Caddy and Gulland 1983). Among the pelagic fishery resources of India, carangids constitute the third most important resource, ranked next to the Indian oil sardine and Indian mackerel (CMFRI 2019). The Indian scad, *Decapterus russelli* is one of the most exploited pelagic fish species in the family Carangidae as it is a low-priced source of animal protein and commonly used as fish live bait (Froese and Pauly 2024). It is widespread in the Indo-Pacific and forms an important fishery resource in India and Southeast Asia (Rohfritsch and Borsa 2005). Most studies on this species have focused on biology, ecology, and taxonomy (Murty 1991; Reuben et al., 1992; Jaiswar et al., 1993; Sunil and Suryanarayanan 1994; Moiseyeva and Zhuk 1995; Raje 1997; Balasubrahmanian and Natarajan 2000; Manojkumar 2005, 2007; Poojary et al., 2010; Sen et al., 2011; Kalhoro et al., 2017), and it is important to study the genetic and genomic traits to resolve taxonomic ambiguities, understand evolutionary traits, and implement conservation strategies.

Molecular genetic markers are consistently used to differentiate between adjacent populations, but several studies have shown that otolith has the potential advantage as a natural population marker (Edmonds et al., 1989; Kalish 1990). The annual (Casselman 1990) and daily (Marshall and Parker 1982; Campana 1983; Campana and Neilson 1985) growth rings are used for the age determination of fishes. In addition, otolith elemental compositions are analyzed to identify and characterize fish populations. Two characteristics of otoliths (Campana et al., 1995) have made them a suitable marker for studying the structure of fish populations. First, otoliths grow throughout the life of the fish and, unlike bones, are metabolically inert, meaning that the elemental composition is unlikely to be resorbed or changed once deposited. Second, the calcium carbonate and trace elements that make up 90% of the otolith appear primarily derived from the water in which they lived. Therefore, the elemental composition of the otolith is thought to reflect that of the water in which the fish lives (Kalish 1989; Fowler et al., 1995). The properties of seawater, both chemical and physical, vary from place to place (Wiesenburg and Little 1987). Therefore, elemental deposition on otoliths largely depends on the

environment to which they are exposed (Radtke and Shafer 1992). Thus, researchers can use otolith microchemistry analysis as a powerful tool to describe fish population structure.

## 1.2 Species description

Indian scad*, D. russelli* is wide spread in the Indo-Pacific from the Red Sea and East Africa to Japan and Australia (Rohfritsch and Borsa 2005; Froese and Pauly 2024). It constitute a major fishery resource in India and South East Asia (Rohfritsch and Borsa 2005). Indian scad mackerel inhabits at a depth range of 40-275 m and most commonly found in coastal and open banks of Indian Ocean (Froese and Pauly 2024). The species has a prolonged spawning season with different studies reported different spawning period which were overlapping along various coast of India suggesting the species as a continuous breeder and prefer to spawn only when the conditions are favorable, which may vary from place to place. The ovarian development is asynchronous and eggs are pelagic and spherical ranging from 0.02 mm-0.97 mm (Poojary et al., 2015). The exact spawning grounds have not been located yet.

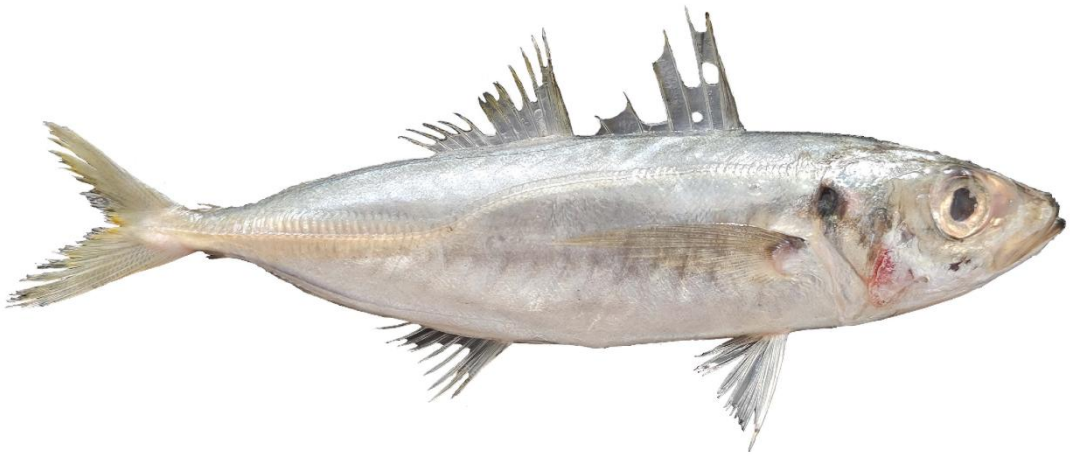

Figure 1.1 *Decapterus russelli* (Rüppell, 1830)

Furthermore, *D. russelli* is a pelagic carnivore showing a pronounced difference in food preference as they increase in size. Planktonic crustaceans (Acetes spp., copepods and other crustaceans) formed the main diet in smaller groups (Manojkumar 2007) and it was replaced by fishes (Lactarius spp., Myctophid spp.) and molluscs (Sreenivasan 1974; Raje 1997; Poojary et al., 2010) in larger groups. Thus the species plays an important role in proper functioning of ecosystem as they avoid direct competition by increasing the number and diversity of prey (Manojkumar 2007; Poojary et al., 2010) as they grow. As a primary carnivore, they have inevitable role in keeping the ecosystem balanced as it is a voracious feeder of planktonic crustaceans (Manojkumar 2007) keeping their population checked to the optimum level not to

over exploit primary producers. They also play a crucial role in maintaining the trophic ecology and integrity of the food web. The large-scale consumption of energy rich planktonic crustaceans (Manojkumar 2007; Poojary et al., 2010) makes this highly vagile species a potential link in the food chain to transfer energy to subsequent trophic levels both in time and place.

## 1.3 The current taxonomic position of *D. russelli* (Froese and Pauly 2024)

Kingdom :Animalia
Phylum :Chordata
Sub phylum :Vertebrata
Super Class :Actinopterygii
Class :Teleostei
Order :Carangiformes
Family :Carangidae
Genus :Decapterus
Species :*Decapterus russelli*

## 1.4 Objectives

Despite its wide spread distribution in the Indian Ocean and high economic importance, genetic data on *D. russelli* is currently scarce. Previous studies on the phylogenetic and evolutionary relationships within the family Carangidae have primarily relied on short mitochondrial gene fragments, which provide limited resolution. A more comprehensive analysis using complete mitochondrial genome data can offer valuable insights into the genetic structure, variability, and evolutionary dynamics of this species—critical information for advancing fishery science.

While the genetic stock structure of *D. russelli* remains unexplored in the Indian Ocean, studies from other regions, such as the Indo-Malay Archipelago (IMA), have examined its geographical structure. Understanding the spatial genetic structure of marine populations is vital for effective fisheries management, as it aids in designing conservation strategies and sustainable exploitation plans. To address these gaps, this research employs advanced bioinformatics and statistical tools to analyze genetic and chemical data, providing answers to key research questions.

The objectives of this study are as follows:

1. Characterization of the complete mitochondrial genome of *D. russelli* and its phylogenetic analysis.
2. Genetic stock structure analysis and historical demography of the Indian scad, *D. russelli* from the Indian Ocean using mitochondrial and nuclear markers.
3. Chemical analysis of otolith for delineating stock structure in *D. russelli* along Indian coast.

This integrated approach will contribute to a deeper understanding of the genetic and ecological dynamics of *D. russelli*, aiding in the development of effective fisheries management strategies.

## 1.5 experimental design

| Objective | Steps | Methodology | Expected outcome |
|---|---|---|---|
| **1. Characterization of the complete mitochondrial genome of *D. russelli* and its phylogenetic analysis** | 1. Specimen collection | • Collection of wild specimens of *D. russelli* from a designated location from the Indian Ocean. | **A well-characterized mitochondrial genome of *D. russelli*, providing insights into its phylogenetic position and evolutionary timeline within Carangidae.** |
| | 2. DNA isolation | • Extraction of total DNA from muscle tissue using a standard protocol (e.g., phenol-chloroform or commercial DNA extraction kits optimized for mitochondrial DNA). | |
| | 3. Genome amplification and sequencing | • **Initial Amplification**: Use of universal primers to target conserved mitochondrial regions (e.g., COI, 12S, 16S rRNA).<br>• **Subsequent Amplification**: Use of specific primers based on initial sequences to cover the entire mitochondrial genome.<br>• **Sequencing**: Sequencing of PCR products using Sanger sequencing. | |
| | 4. Genome assembly and annotation | • Assembling of mitochondrial genome sequences using bioinformatics tools.<br>Genome annotation to identify protein-coding | |

| | | | |
|---|---|---|---|
| | | genes, rRNA, tRNA, and non-coding regions (e.g., using MITOS, ARWEN). | |
| | 5. Comparative and phylogenetic analysis | • Retrieval of all the available mitochondrial genomes of the family Carangidae from GenBank.<br>• Performing multiple sequence alignment (e.g., with Clustal W in MEGA).<br>• Constructing a phylogenetic tree to establish *D. russelli*'s evolutionary position using MEGA or MrBayes.<br>• Molecular clock analysis to estimate divergence times. | |
| **2. Genetic stock structure analysis and historical demography of the Indian scad, *D. russelli* from the Indian Ocean using mitochondrial and nuclear markers.** | 1. Sampling | • Collection of samples from multiple locations from the east and west coasts of India, ensuring sufficient geographical distance between locations to capture genetic variability. | **Insights into the genetic stock structure and demography of *D. russelli*, providing valuable data for fisheries management and conservation.** |
| | 2. DNA Isolation and marker selection | • Extraction of DNA from each sample.<br>• Selection of suitable genetic markers- both mitochondrial and nuclear markers for analysis. | |
| | 3. Primer designing and amplification | • Designing of new primers and PCR amplification of the targeted regions | |

| | | | |
|---|---|---|---|
| | 3. Sequence analysis and data retrieval | • **Sequence Generation**: Sequencing of the mitochondrial and nuclear markers for each sample.<br>• **Data Retrieval for Comparative Analysis**: Retrieval of available sequences of *D. russelli* from IMA for comparison and analyze sequences to identify possible genetic breaks. | |
| | 4. Genetic structure analysis | • **Population Structure**: Use of software such as Arlequin to assess population genetic parameters, including F-statistics (e.g., F_ST, AMOVA) to detect population differentiation.<br>• **Genetic Diversity Analysis**: Use MEGA or DnaSP to calculate genetic diversity indices (e.g., haplotype diversity, nucleotide diversity) and compare diversity across populations. | |
| | 5. Historical demographic analysis | • Use DnaSP to analyze historical demography (e.g., mismatch distributions, neutrality tests) to identify demographic changes like population expansions or bottlenecks. | |
| **3. Chemical analysis of otoliths for delineating stock structure in *D. russelli*** | 1. Sample collection and otolith extraction | • Collection of otolith samples from fish at various locations from the east and west coasts of India, ensuring sufficient geographical distance between locations. | **Identification of stock-specific chemical signatures in otoliths that** |

| | | | |
|---|---|---|---|
| | | • Extraction and sterilization of sagittal otoliths according to standardized protocols. | **corroborate genetic findings, enhancing the robustness of stock delineation efforts for *D. russelli*.** |
| | 2. Chemical analysis of otoliths | • Analysis of the elemental composition of otoliths using inductively coupled plasma-optical emission spectrometry (ICP-OES).<br>• Measuring the whole sagittal elemental composition to reflect environmental water signatures that vary by location. | |
| | 3. Data Analysis | • Performing statistical analysis (ANOVA, MANOVA, and discriminant analysis) to assess variations in elemental composition across locations.<br>• Comparing the chemical data with genetic data to corroborate findings from genetic stock structure analysis. | |

# CHAPTER 2

# REVIEW OF LITERATURE

## 2.1 Previous research on the targeted species

The phylogenetic relatedness between the carangids has been extensively studied using comparative morphological (Suzuki 1962; Smith-Vaniz and Staiger 1973; Smith-Vaniz 1984, 1986; Gushiken 1988; Springer and Smith-Vaniz 2008; Hilton et al., 2010) and molecular tools (Reed et al., 2002; Cárdenas et al., 2005; Betancur-R. et al., 2013; Near et al., 2013; Rabosky et al., 2013). Although there has been a discourse on monophyly (Smith-Vaniz 1984, 1986; Gushiken 1988; Springer and Smith-Vaniz 2008; Hilton et al., 2010) vs. paraphyly (Near et al., 2013; Rabosky et al., 2013) of carangids, the congruent topologies of phylogenetic trees constructed using DNA sequences suggested paraphyly of carangids if Echeneoids were not included (Betancur-R. et al., 2013; Near et al., 2013; Rabosky et al., 2013; Santini and Carnevale 2015). Carangids can be considered as promising candidates for evolutionary studies, as they have a wide diversity in terms of habitat (marine, brackish water, freshwater), morphology (having variable body size; less than 20 cm to ~2 m), and shape (deep-bodied, streamlined). Some species of remoras live even as commensals on large pelagic vertebrates (Britz and Johnson 2012). The phylogeographic study inferred from nuclear gene supermatrix suggested a terminal cretaceous crown age for carangids with major lineages diversifying during the Paleogene (Near et al., 2013). A late Eocene age has also been proposed for the crown carangids (Betancur-R. et al., 2013). But densely sampled molecular studies indicated a Cenomanian stem age of the Carangoids which had undergone a significant diversification during the late cretaceous (Rabosky et al., 2013; Santini and Carnevale 2015). The subfamily Naucratinae originated early in the Paleocene (Swart et al., 2015), Trachinotinae date back to the early Oligocene and the remaining Carangoid lineages including the morphologically heterogeneous group, Caranginae comprising the genus *Decapterus* appear to have originated during the middle Eocene (Santini and Carnevale 2015). The genus *Decapterus* originated in the upper Miocene (Bannikov 1987). The genetic distance between the genus *Decapterus* was significantly greater than that between *Trachurus* (Kijima 1988), suggesting that *Decapterus* arose long before its sister group *Trachurus*, making them the most divergent species group. The morphology of the genus *Decapterus* includes multiple forms such as long-bodied, cylindrical, mackerel-like with a small mouth, reduced teeth and reduced scutes on the straight part of the lateral line, suggesting adaptations to the wide ranging off-shore life (Nichols 1942).

Although Zhu et al (Zhu et al., 2008) analysed the phylogenetic relationships of carangids using the control region, there is a gap in knowledge regarding the mutational pattern and the non-

random distribution of these mutations between the conserved motifs and their flanking regions. There is also a lack of knowledge about the structure and variability of tRNA genes in carangids and the frequency of occurrence of G-U wobble base pairs, the fundamental building block of RNA structure critical for RNA function in various biological systems.

*D. russelli* has been extensively studied mainly on its biology and population dynamics ( Murty 1991; Raje 1997; Balasubrahmanian and Natarajan 2000), length- weight relationship (Jaiswar et al., 1993), food and feeding habit (Poojary et al., 2010), reproductive biology (Sunil and Suryanarayanan 1994; Moiseeva and Zhuk 1995; Manojkumar 2005) and stock status (Reuben et al., 1992; Kalhoro et al., 2017). Extensive investigation on its geographical structure have also been undertaken from the Indo-Malay Archipelago (IMA) (Perrin and Borsa 2001; Rohfritsch and Borsa 2005). Previous studies have been reported that two populations of scad mackerels seasonally enter into Java Sea, one during East monsoon, from Flores Sea and Makassar strait and another during West monsoon from Indian Ocean and Sunda strait (Hardenberg 2016). In addition, it also suggested the possibility of entering a third population into the Java Sea from the South China Sea during West monsoon. The migration and distribution of Indian scad mackerel can be ascribed to the salinity variation in relation with the monsoon cycle in the IMA (Hardenberg 2016). Two putative lineages were distinguished in *D. russelli* from IMA based on sequence polymorphism of the mitochondrial *Cyt b* gene (Perrin and Borsa 2001). The two lineages reportedly had shown spatial heterogeneity in their distribution. Later on, Rohfritsch and Borsa (2005) detected 3 mitochondrial lineages of *D. russelli* from IMA based on the combined result from mitochondrial and nuclear markers. One of the haplogroup of Perrin and Borsa (2001) appeared to consist of 2 separate lineages in Rohfritsch and Borsa (2005). They could also observe the co-occurrence of some mitochondrial lineages, indicative of recent secondary contact between once isolated populations or admixture in samples of cryptic species (Hoarau and Borsa 2000).

Genetic information on this species from the Indian Ocean is still scarce. Except for one study utilizing truss morphometric analysis (Sen et al., 2011), *D. russelli* from the Indian Ocean are less investigated than their Indo-Malay Archipelago counterparts to decode their geographical structure using molecular markers. Sen et al. (2011) employed 23 variables to differentiate between populations and identified separate stocks from East and West coasts of India. The genetic basis for this observed phenotypic discreteness needs to be established further for effective management recommendations for the contemporary species in different geographical

areas across Indian Ocean. Hence, we investigated the genetic population structure of Indian scad mackerel collected from five locations using mitochondrial and nuclear markers. Further, in order to determine any genetic break between the populations of *D. russelli*, we performed a comparative study by expanding the geographic area to IMA. In addition, we also aimed to analyse the stock identity of this species by employing an environmentally influenced marker, i.e., otolith. Since the species has a high economic value, and it is widely harvested, the stock identity of such species will provide adequate information to implement effective management strategies for the sustainable utilization of the fishery resources.

## 2.2 Mitogenome characterization

mtDNA is an important model system in fisheries and aquaculture research for investigating the genome structure, molecular evolution, and phylogeny (Curole and Kocher 1999; Podsiadlowski et al., 2007). By characterizing the complete mitochondrial genome among fish taxa, we can analyse gene arrangement and distribution, nucleotide composition, protein coding information, transfer RNA (tRNA), ribosomal RNA (rRNA) genes and non-coding regions (Yang et al., 2018) and more importantly to provide these data for further studies in fisheries science.

Genomic information is considered to be reliable for the efficient implementation of strategies for studying evolutionary relationships, phylogeography and phylogeny (Harrison 1989; Excoffier et al., 1992; Moritz 1995; Miya et al., 2005, 2013; Jacobsen et al., 2012). Animal mitochondrial DNA (mtDNA) offers distinct advantages over other genes for phylogenetic analysis. The mitochondrial genomes of fish have a similar organization to that of other vertebrates (Kartavtsev et al., 2007). Continuous technological advancements have enabled the rapid sequencing of complete mitochondrial genomes. New genome sequences are being published at a rate of more than one per month. These longer sequences allow us to reconstruct relationships between more divergent lineages (Curole and Kocher 1999). In fish, the first report on the mitochondrial genome was from the freshwater loach (*Crossostoma lacustre*; GenBank Accession number: M91245) (Tzeng et al., 1992).

Soon after mtDNA sequences from various species became available, mitochondrial protein sequence comparisons revealed deviations from the standard genetic code and differences in codon usage (Osawa et al., 1992). Utilizing a simplified decoding mechanism that permits

translation of all codons with less than 32 tRNA species in accordance with Crick's wobble theory is another unique feature of the mitochondrial genetic system (Taanman 1999). In addition, unusual structures have observed in certain vertebrate tRNAs. The serine tRNA (tRNA-Ser (AGY)) lacks a Dihydrouridine (DHU) arm, which is a common feature found in all vertebrates (Jang et al., 2015; Zhang et al., 2019).

The different regions of the mtDNA undergo mutations at different rates. The protein coding genes (PCGs), tRNA and rRNA have a relatively low mutation rate because they are subject to strong purifying selection (Jacobsen et al., 2016). In contrast, the control region evolves at a high rate both within (Fauron and Wolstenholme 1980; Aquadro and Greenberg 1983; Greenberg et al., 1983) and between (Fauron and Wolstenholme 1980; WM 1985) species compared to the other parts of the mitogenome, and these high rates of sequence divergence are mostly localized in the variable flanking region, which is adjacent to the conserved sequence motifs (Brown et al., 1982; Foran et al., 1988; Saccone et al., 1991).The extensive comparative studies in fish (Sebastian et al., 2020) have demonstrated the ability of the control region to form stable secondary structures and the existence of selective constraints for their maintenance.

Although the gene content of vertebrate mtDNA is almost constant, the relative position of these genes in the genome varies between major taxonomic groups (Curole and Kocher 1999). The mechanisms underlying these rearrangements are not well understood, but it appears that tRNA genes change position more frequently than rRNAs and PCGs (Cantatore et al., 1987; Macey et al., 1997; Arndt and Smith 1998; Mukundan et al., 2020). Such rearrangements can be used as cladistic characters to examine relationships between higher level taxa (Smith et al., 1993).

## 2.3 Population structure analysis

All organisms undergo mutations resulting in genetic variations as a result of normal cellular processes (Behjati et al., 2014) or interactions with their environment (King et al., 2006). These variations improve the organism's ability to adapt to the changing environment and are necessary for its survival (Loewe and Hill 2010). These differences between individuals may lead to genetic differentiation at the level of species, populations and even higher-order taxa (Vijay et al., 2017). The genetic diversity data has numerous applications in research in the

field of evolution, conservation and management. Molecular genetic markers have the powerful ability to identify these variations at the genetic level (Duran et al., 2009) and therefore can provide precise and appropriate information in the context of individuals, species and populations at the genetic level.

Many studies use mtDNA to identify population divergence in marine fishes ( Liu et al., 2007; Canino et al., 2010; Veríssimo et al., 2010). It is widely known why mtDNA has become the preferred marker. First, since mtDNA exists in numerous copies within the cell (Curole and Kocher 1999), it is relatively easy to amplify experimentally. Next, animals' mitochondrial gene content is highly preserved, with relatively few duplications and very short intergenic segments (Gissi et al., 2008). Finally, Because of high rate of mutation, mitochondrial DNA is highly variable in natural populations and thus can provide information on population history over brief periods (Galtier et al., 2009). These reasons make mitochondrial DNA a popular marker for assessing variation at intraspecific and interspecific levels (Avise et al., 1986, 1998) allowing for the analysis of population relatedness and the subsequent clarification of its genetic structure.

MtDNA has been extensively used for stock structure analyses in a variety of vertebrates including fishes (Avise et al., 1986; Graves et al., 1992; Chow 1993; Gold et al., 1993; Heist and Gold 1999; Sukumaran et al., 2016), birds (Baker and Marshall 1997; Greenberg et al., 1998; Milà et al., 2000; Zink et al., 2000), mammals (Menotti-Raymond and O'Brien 1993) and reptiles (Avise et al., 1998; Serb et al., 2001; Riberon et al., 2002; Shanker et al., 2004). There are many regions in the mitochondrial genome such as the Cytochrome oxidase subunit I (*COI*) cytochrome b gene (*Cyt b*), the D-loop/control region, *ATPase6/8* and *NADH* that are suitable for studying the genetic structure of the population. *COI* gene has been used in many fish species to investigate the genetic structure of population (Ali et al., 2014; Tan et al., 2015; Thirumaraiselvi and Thangaraj 2015; Modeel et al., 2023). Several studies on mtDNA have focused on its major noncoding region i.e., control region, or D loop, located between the tRNA-Thr and tRNA-Phe genes due to its complex structure and supposedly rapid rate of evolution (Cheng et al., 2012; Kumar et al., 2012). Many others focused on the *Cyt b* gene to study the genetic structure of the population (Reed et al., 2001, 2002; Rohfritsch and Borsa 2005; Sun et al., 2012; Akib et al., 2015). The *NADH* gene ( Gasser et al., 1999; Caballero et al., 2015; Tadayon et al., 2015) can also provide information about the genetic structure of population. In addition, many other studies have employed the *ATPase 6/8* regions for the

analysis of phylogeny as well as phylogeography in several fish species (Chow and Ushiama 1995; Dammannagoda et al., 2008; Vergara-Chen et al., 2009; Habib et al., 2012). By using such regions with different degree of mutation rate, we could get a comprehensive overview of the molecular relationship by directly analyze the nucleotide sequences.

Sometimes, mtDNA sequence phylogeny and haplotype frequency data are insufficient to support genetic differentiation or cryptic speciation. In that case, employing nuclear markers to broaden the study will improve the accuracy of mitochondrial phylogeography of species under question. Thus, the inclusion of nuclear markers and the genealogical data from the nuclei loci are essential in order to obtain a comprehensive understanding of the evolutionary potential and history of populations. Random nuclear markers such as Randomly Amplified Polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), Amplified Fragment Length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLP) amplify products of less functional part of the genome and the regions targeted by these markers may contain more mutations (Williams et al., 1990) and also they can detect high levels of DNA polymorphism (Williams et al., 1990). In addition, these markers have found the widest application in genetic variation analyses below the species level, especially in studies of population structure and differentiation (Chong et al., 2000; Küçük 2010; Song et al., 2010; Han et al., 2015; Zhang et al., 2012) and genetic variation within populations (Wang et al., 2000; Liu et al., 2005; Rahman et al,, 2009; Popoola et al., 2014).

In addition to random markers, there are specific nuclear DNA markers that can be targeted to specific regions in the genome. In the nuclear genome, certain DNA segments exist as tandem repeats of tens, hundreds, or thousands (Legendre et al., 2007). It has been proposed that the two major classes of repetitive DNA found in individuals are minisatellite and microsatellite DNA (Ramel 1997). Of these, the most abundant repeat found in the genome, the microsatellite loci (Debrauwere et al., 1997) are widely used in the field of population studies to assess the genetic structure of vertebrates and invertebrates. In addition, other nuclear markers such as Single Nucleotide Polymorphism (SNPs) are becoming a central focus in the development of molecular markers as they represent the most abundant polymorphism in both coding and non-coding regions of an organism's genome (Garvin et al., 2010), adaptable to automation (Duran et al., 2009), and have the ability to reveal hidden polymorphism not detected with other markers and technologies (Liu 2008). Another progress in the development of nuclear DNA marker is the Expressed Sequence Tags (ESTs) (Adams 1980). This marker can answer

questions at the genetic and genomic levels and can even be used to study nonmodal organisms that lack other sequence resources (Tanya and Kumar 2010).

Researchers typically assess the population structure of fish species using genetic markers such as mitochondrial or nuclear markers. In some studies, both otolith and genetic markers have been used in parallel to delineate the characteristics fish populations (Ashford et al., 2006; Bradbury et al., 2008; Gibson et al., 2008; Barnett-Johnson et al., 2010; Woods et al., 2010; Collins et al., 2013). Use of Chemical tracers enable us to determine whether populations are connected on an ecological time scale (Radtke and Shafer 1992), whereas, genetic analyses can be used to determine whether they are connected on an evolutionary time scale (Broughton et al., 2013; Ruan et al., 2020). Therefore, by using multiple types of markers, researchers can determine whether populations are connected at multiple time scales (Rubenstein and Hobson 2004; Leis et al., 2011). However, choosing a suitable marker system for a population genetics study requires careful consideration of factors like sensitivity for the research questions being asked and practical measures for data collection.

# CHAPTER 3

## Characterization of the complete mitochondrial genome of *D. russelli* and its phylogenetic analysis

## 3.1 Introduction

Mitochondria contain their own genome, a circular DNA molecule present in several copies per organelle. The mitochondrial genome is essential for life in almost all eukaryotes and repertoire of encoded genes is extremely conserved. mtDNA is an important model system in aquaculture research for investigating the genome structure, molecular evolution, and phylogeny (Curole and Kocher 1999; Podsiadlowski et al., 2007). By the characterization of complete mitochondrial genome among fish taxa, we can analyze the gene arrangement and distribution, nucleotide composition, information about protein coding, tRNA, rRNA genes, and non-coding regions (Yang et al., 2018) and more importantly to provide these data for further studies in fishery science.

A precise phylogeny is necessary to fully comprehend the evolution of various species. To adequately resolve this higher-level relationship, longer DNA sequences are required (Stepien and Kocher 1997; Miya and Nishida 2000). The novel methods for sequencing the entire mitogenome of fish (Miya and Nishida 1999) and the explicit demonstration of the phylogenetic utility of this mitogenomic data (Miya and Nishida 2000) enabled us to address several controversial questions in the field of fish phylogenetics. In addition, the mitogenomic data can also be used to address inter and intraspecific relationship in addition to resolving higher level relationships. Furthermore, various uncertainties in divergence time estimation based on fossils can be circumvented by the use of molecular data obtained from mitogenomic data (Azuma et al., 2008) since the fossils of many fish lineages are not considered well preserved (Benton and Donoghue 2007).

A comparative mitogenomic analysis between fish taxa allows us to compare the structural properties of the mitogenome and determine whether these properties are conserved with respect to gene order, tRNA, rRNA secondary structures, and the control region. This allows us to answer questions such as whether the preservation of gene order is a consequence of certain constraints or whether it is the result of common ancestry. It has been discerned that most vertebrates have an identical mitochondrial gene order (Miya and Nishida 2015), and deviations from this structural property can be used as markers for assessing phylogenetic relatedness.

The size of vertebrate mitochondrial genome is approximately 15-20 kb and consists of 13 PCGs, 22 tRNA genes, 2 rRNA genes and 1 non coding region (control region) (Boore 1999). Fish mitochondrial genomes have a similar organization to that of other vertebrates (Kartavtsev et al, 2007). In fish, first report on the mitochondrial genome (Tzeng et al., 1992) was from freshwater loach (*Crossostoma lacustre*; GenBank Accession number: M91245).To date, the complete mitochondrial genome of 36 species of the family Carangidae are known: (*Alectis ciliaris* (Li et al., 2016a), *Alectis indica*, *Alepes djedaba* (Li et al., 2020), *Alepes kleinii* (Li and Zou 2016), *Atule mate* (Li et al., 2016b*),Carangoides armatus* (Miya et al., 2003), *Carangoides bajad* (not published), *Carangoides equula* (Zou et al., 2016), *Carangoides malabaricus* (Li et al., 2016c), *Carangoides plagiotaenia* (Kaure et al., 2021)*,Caranx ignobilis* (not published) *Caranx melampygus* (Miya et al., 2003), *Caranx tille* (Ma et al., 2016), *Decapterus macarellus* (Zou et al., 2016), *Decapterus macrosoma* (Li et al., 2016d), *Decapterus maruadsi* (Li et al., 2016e), *Decapterus tabl* (Li et al., 2020), *Elagatis bipinnulata* (Ma et al., 2107a), *Gnathanodon speciosus* (Jiang et al., 2022), *Megalaspis cordyla* (Li et al., 2016f), *Parastromateus niger* (Chen et al., 2016), *Pseudocaranx dentex* (Li et al., 2021), *Trachurus japonicas* (not published), *Trachurus trachurus* (Takashima et al., 2006), *Selar crumenophthalmus* (Li et al., 2016g), *Seriola dumerili*, *Seriola lalandi*, *seriola quinqueradiata* and *Seriola rivoliana* (Iguchi et al., 2012), *Selaroides leptolepis* (not published), *Seriolina nigrofasciata* (Liu et al., 2016), *Trachinotus blochii* (Zhang et al., 2016a), *Trachinotus ovatus* (Xie et al., 2015), *Trachinotus Carolinus* (Zhang et al., 2016b), *Uraspis helvola* (not published), *Uraspis secunda* (Ma et al., 2017b).

## 3.2 Review of Literature

mtDNA represents only a tiny fraction of organismal genome size, yet it has generated considerable research interest as a popular marker of molecular diversity over the last three decades. The reasons for the adoption of mtDNA as a marker of choice are well known. Experimentally, mtDNA is relatively easy to amplify because it appears in multiple copies in the cell. Mitochondrial gene content is strongly conserved across animals, with very few duplications, very short intergenic regions (Gissi et al., 2008), and the absence (or very low level) of recombination (Elson and Lightowlers 2006). Numerous researches have established that these mitochondrial genes are popular genetic marker for evolutionary studies (Satoh et al., 2016; Damerau et al., 2018), because they are effectively single copy, they show clonal pattern of inheritance and reduced effective population size (Curole and Kocher 1999).

However, such short mitochondrial gene fragments exhibit limitations in resolving complicated phylogenetic relationships in many fish lineages ( Stepien and Kocher 1997). The additional informative sites from longer DNA sequences (e.g., mitochondrial genomes) allow these deeper branches and higher-level relationships to be more fully resolved (Miya and Nishida 2000).

The gene content of vertebrate mtDNA is nearly constant. However, the relative position of these genes in the genome varies among major taxonomic groups. The mechanisms that underlying these rearrangements are not well understood, but it is seen that tRNA genes change their position more frequently than either rRNAs or PCGs (Cantatore et al., 1987; Macey et al., 1997; Arndt and Smith 1998). Such rearrangements can be used as cladistic characters for studying relationships among higher level taxa (Smith et al., 1993).

The research on mitochondrial genomes is one of the ground breaking discoveries in the history of scientific world. In 1981, Anderson et al. (1981) and Bibb et al. (1981) sequenced the whole mtDNA of human and mouse respectively. These were the first non-viral genome to be completely sequenced. Soon after, the complete mitochondrial sequences of bovine (Anderson et al., 1982) and other animals were decoded which along with many other deposited partial mitochondrial DNA sequences fuel the field of comparative mitogenomics. By the end of 1990s, dozens of animal mtDNA (Boore 1999) as well as complete mitochondrial sequences from land plants (Oda et al., 1992), algae (Vahrenholz et al., 1993), fungi (Cummings et al., 1990), and other protists (Kairo et al., 1994) had been deciphered.

Continued technological advancements have made possible the rapid sequencing of complete mitochondrial genomes which included new methods of fast and efficient isolation techniques, amplification, assembly and annotation of these longer DNA sequences (DeSalle et al., 1993; Cheng et al., 1994; Pollock et al., 2000; Wyman et al., 2004; Burger et al., 2007). In addition, enhanced long-range polymerase chain reaction (Yamauchi et al., 2004) and improved sanger sequencing technology (Metzker 2005) made the whole procedure of whole mitogenome amplification and sequencing simple and affordable even among smaller laboratory groups.

Currently majority of the mtDNA (more than 90%) being sequenced and deposited in GenBank come from metazoans (Smith 2016). The widespread sequencing of metazoan mitochondrial DNA helped the scientific community to unravel certain interesting genomes including that of

lice (Shao et al., 2012). The research on mitochondrial DNA has contributed a lot to shape our knowledge about the origin, diversity and complexity of eukaryotic life (Gray 2012).

## 3.3 Materials and Methods

### 3.3.1 Sample collection

The wild specimen of *D. russelli* was collected from Kochi, Kerala (9°56'19"N 76°15'45"E) from trawl and purse seines. A caudal fin base sample of a single individual was preserved in 95% ethanol for DNA extraction and further analysis.

### 3.3.2 DNA extraction and PCR amplification

Genomic DNA was isolated using standard phenol – chloroform - isoamyl alcohol extraction cycle (Sambrook and Russell 2001). The extracted DNA was then eluted in T.E buffer (1X) and stored at -20$^0$C for subsequent use as a PCR template in further analysis. 40 locations were amplified via PCR to determine the complete mitochondrial genome of *D. russelli.* The primers for the 40 locations include 10 universal primers that were designed by Ward et al. (2005); Palumbi (1996); Infante et al. (2006); Cheng et al. (2012) and Sivasundar et al. (2001) and 30 custom designed primers (Table 3.1). The sequenced products of initial amplifications using mitochondrial universal primers then served as the templates for subsequent reaction using specific primers. The new primer pairs were designed with reference to the aligned complete mitogenome sequences of 36 species of Carangidae using primer 3 Software (version 0.4.0) (http://frodo.wi.mit.edu/primer3/) (Rozen and Skaletsky 1999). PCR amplifications were carried out in Biorad T100 thermocycler (Biorad, USA). PCR conditions were designated for different regions of mitochondrial DNA are shown in Table 3.2. The products were then sequenced completely in both directions using sanger sequencing.

### 3.3.3 Sequence Analysis

The sequencing results were manually proofread by AB sequence scanner then the complete mitochondrial genome sequence of *D. russelli* was assembled using the software MEGA X (Kumar et al., 2018). The mitogenomic sequence identity of *D. russelli* was verified by blast search and the sequences were annotated based on the alignments with other Carangidae acquired from the GenBank database. The organization of the mitochondrial genome and the order of the genes were initially determined by MitoFish database (Iwasaki et al., 2013) and

later verified manually by alignment with the sequences of other closely related species. The protein coding genes and their codon usage were summarized with MEGA X (Kumar et al., 2018). The tRNA genes and their secondary structures were predicted with tRNA scan SE 2.0 (Chan et al., 2021) with default parameters coupled with ARWEN software (Laslett and Canbäck 2008). The secondary structure of conserved sequence motifs of the control region was inferred using Mfold web server (Zuker 2003).

Table 3.1 PCR primers designed for the characterization of complete mitochondrial genome of *D. russelli*

| Primer | Nucleotide sequence | Primer | Nucleotide sequence |
|---|---|---|---|
| 12S_16S_864 F | AGCAGTAAGCAGGAAATAGAGTG | COI_Cytb_10379R III | CCTGCRCTTGCTTCRCAH |
| 12S_16S_2235 R | CTGGAAAGAGGAGACAGTTAAGC | COI _Cytb_10220F IV | CATTCCATCGCTACCACCTT |
| 12S_DLOOP_16471 F | TGGTGGAAAGATATTGGAAGG | COI _Cytb_11831R IV | GGCTGTCCACCCTCAGATTA |
| 12S_DLOOP_618 R | ATGTTAAGCACCGCCAAGTC | COI _Cytb_12387F V | CATACACTCGGACCCGTACA |
| 16S_COI_ 2635 F | AGCCGCTATTAAGGGTTCGT | COI_ Cytb_13169R V | TAGGCAGGAGGAGGTGAATG |
| 16S_COI_ 4250 R | DGGRATRATGGCTAGGGTRT | Cytb_COI_13023F | ARCTTGCYTTCCTCCAYATY |
| 16S_COI_3795F II | ACGACCAGCTTATGCACCTT | Cytb_COI_14633R | GGCAAGGAAGAGTCCAGTGA |
| 16S_COI_5207 R II | GCCCTTGGTCTTGTRYTAWC | Cytb_COI_11584F II | AACTHATRATCATCACATCCCTD |
| 16S_COI_4995F III | ACTTTTACCTCCGCCTCTCA | Cytb_COI_13187R II | GTTAGGCAGGAGGAGGTGAA |
| 16S_COI_5754R III | TGGGCCGTAACAATTACGTT | Cytb_COI_9309R VI | CCAGACGCTAAAAGGACTGC |
| COI_Cytb_6177F | GCTGCTGGTATTACAATGCTTC | Cytb_COI_14323F VII | AGTGCCGCAAAGTAAGGAGA |
| COI_Cytb_7801R | DGGGACTGCRTCRACTTTYA | Deru Cytb_07F | AATCTCCGTAAAACCCACCC |
| COI_Cytb_7639F II | TGGTCCCTACACAGGACCTAA | Deru Cytb_1R | AAAAACTAGGAATAGGAGGAAGT |
| COI_Cytb_9049R II | GGGCTGTDGTAAGGGTRACRAGR | Cytb_Dloop_15135F | TCCACCCATACTTTTCCTACAAA |
| COI_Cytb_8777F III | CACAACCATCCTTCTGTTTCTTC | Cytb_Dloop_R_ 15766 | TCGCGCAGAGAATAGTTTAATG |

Table 3.2 PCR Conditions and the primer pairs used for the amplification of different regions of mitochondrial DNA of *D. russelli*. Shaded regions represent the primers designed for this study.

| Region | Primer used | | Initial denaturation | 32 cycles of | | | Final Extension |
|---|---|---|---|---|---|---|---|
| | Forward | Reverse | | Denaturation | Annealing | Extension | |
| 16S rRNA | 16SarL | 16SbrH | 94$^0$C 4 min | 94$^0$C 30 sec | 55$^0$C 30 sec | 72$^0$C 1 min | 72$^0$C 10 min |
| ATP6/8 | ATP8.2L8331 | CO111.2H9236 | 94$^0$C 4 min | 94$^0$C 30 sec | 55$^0$C 30 sec | 72$^0$C 1 min | 72$^0$C 10 min |
| D loop | D_Thr_F | Dloop_Phe_R | 94$^0$C 4 min | 94$^0$C 30 sec | 55$^0$C 30 sec | 72$^0$C 1 min | 72$^0$C 10 min |
| 12S rRNA | 12S1 | 12S2 | 94$^0$C 4 min | 94$^0$C 30 sec | 45.8$^0$C 30 sec | 72$^0$C 1 min | 72$^0$C 10 min |
| CO1 | FishF1 | FishR1 | 94$^0$C 4 min | 94$^0$C 30 sec | 53$^0$C 30 sec | 72$^0$C 45 sec | 72$^0$C 7 min |
| Cyt b | Deru Cytb_07F | Deru Cytb_1R | 94$^0$C 4 min | 94$^0$C 30 sec | 55.4$^0$C 30 sec | 72$^0$C 1 min 10 sec | 72$^0$C 10 min |
| Between 12SrRNA and 16S rRNA | 12S_16S_864 F | 12S_16S_2235 R | 94$^0$C 4 min | 94$^0$C 30 sec | 60.4$^0$C 30 sec | 72$^0$C 1 min 10 sec | 72$^0$C 7 min |
| Between 12SrRNA and D loop | 12S_DLOOP_16471 F | 12S_DLOOP_618 R | 94$^0$C 4 min | 94$^0$C 30 sec | 55.4$^0$C 30 sec | 72$^0$C 45 sec | 72$^0$C 7 min |
| Between 16S rRNA and CO1 | 16S_COI_ 2635 F | 16S_COI_ 4250 R | 94$^0$C 4 min | 94$^0$C 30 sec | 56$^0$C 30 sec | 72$^0$C 1 min | 72$^0$C 10 min |
| | 16S_COI_3795F II | 16S_COI_5207 R II | 94$^0$C 4 min | 94$^0$C 30 sec | 46$^0$C 30 sec | 72$^0$C 2 min | 72$^0$C 10 min |
| | 16S_COI_4995F III | 16S_COI_5754R III | 94$^0$C 4 min | 94$^0$C 30 sec | 54$^0$C 30 sec | 72$^0$C 1 min | 72$^0$C 7 min |
| Between CO1 and Cyt b | COI_Cytb_6177F | COI_Cytb_7801R | 94$^0$C 4 min | 94$^0$C 30 sec | 58$^0$C 30 sec | 72$^0$C 2 min | 72$^0$C 10 min |
| | COI_Cytb_7639F II | COI_Cytb_9049R II | 94$^0$C 4 min | 94$^0$C 30 sec | 57$^0$C 30 sec | 72$^0$C 1.50 min | 72$^0$C 10 min |
| | COI_Cytb_8777F III | COI_Cytb_10379R III | 94$^0$C 4 min | 94$^0$C 30 sec | 55.5$^0$C 30 sec | 72$^0$C 2 min | 72$^0$C 10 min |
| | | Cytb_COI_9309R VI | 94$^0$C 4 min | 94$^0$C 30 sec | 62$^0$C 30 sec | 72$^0$C 45 sec | 72$^0$C 7 min |
| | COI _Cytb_10220F IV | COI _Cytb_11831R IV | 94$^0$C 4 min | 94$^0$C 30 sec | 60$^0$C 30 sec | 72$^0$C 2 min | 72$^0$C 10 min |
| | COI _Cytb_12387F V | COI_ Cytb_13169R V | 94$^0$C 4 min | 94$^0$C 30 sec | 57.7$^0$C 30 sec | 72$^0$C 1 min | 72$^0$C 7 min |
| | Cytb_COI_13032F | Cytb_COI_14633R | 94$^0$C 4 min | 94$^0$C 30 sec | 52$^0$C 30 sec | 72$^0$C 2 min | 72$^0$C 10 min |
| | Cytb_COI_14323F VII | | 94$^0$C 4 min | 94$^0$C 30 sec | 59$^0$C 30 sec | 72$^0$C 45 sec | 72$^0$C 7 min |
| | Cytb_COI_11584F II | Cytb_COI_13187R II | 94$^0$C 4 min | 94$^0$C 30 sec | 47.6$^0$C 30 sec | 72$^0$C 2 min | 72$^0$C 10 min |
| Between Cytb and D loop | Cytb_Dloop_15135F | Cytb_Dloop_R_15766 | 94$^0$C 4 min | 94$^0$C 30 sec | 52.3$^0$C 30 sec | 72$^0$C 45 sec | 72$^0$C 7 min |

The whole mitogenome map of the species was graphically visualized by organellarGenomeDRAW version 1.3.1 (Greiner et al., 2019). The nucleotide composition and base skew analysis of whole mitogenome were performed manually in Microsoft Excel based on the formula, AT skew= [A-T] / [A+T]; GC Skew= [G-C] / [G+C]. The codon usage and relative synonymous codon usage (RSCU) were determined using MEGA X (Kumar et al., 2018) by assuring their codon frame. The Watson-crick, wobble and mismatched base pairing in various stem regions of tRNA (acceptor (AA) stem, anticodon (AC) stem, dihydrouridine (DHU) stem and pseudouridine (TΨU) stem) were checked manually by observing their secondary structure obtained from tRNA scan SE (Chan et al., 2021). The synonymous substitution per synonymous sites (Ka) and non synonymous substitution per non synonymous sites (Ks) were calculated using the software package DnaSPv5.0 (Librado and Rozas 2009). The synonymous to non synonymous substitution rates for each PCG were calculated manually by using the formula Ka/Ks. The presence of central conserved sequence block (CSB-F, -E, -D) conserved sequence block (CSB-1, -2, -3), and the palindromic sequence motifs were determined by the alignment of control region of 36 Carangidae using clustal W and their sequences were defined by manual searching. The 37 mitogenomes of Carangidae were compared by using CG view comparison tool (CCT) (Grant et al., 2012) with *D. russelli* as the reference sequence. Genes were assigned by Clusters of Orthologous Groups (COG) using the CG view comparison tool (CCT) and BLAST was used to align other genomes to that of *D. russelli*.

### 3.3.4 Phylogenetic Analysis

All available mitogenome sequences of other carangid species were retrieved from GenBank to find out the relative position of *D. russelli* among carangids and to delineate its phylogenetic relationship. *L. crocea* (GenBank accession number NC011710) was taken as an outgroup. Sequences included in the study and their accession numbers are given in Table 3.3. The 13 PCGs were aligned using Clustal W and translated into amino acid sequences in MEGA X (Kumar et al., 2018). For the subsequent phylogenetic analysis, multiple alignments were generated for the 13 concatenated protein-coding sequences of *D. russelli* and 36 other Carangidae species. The phylogenetic relationship was inferred by constructing a tree based on Bayesian inference (BI) and maximum likelihood (ML) methods. The best model, GTR+G+I, was selected using Akaike

Information Criterion (AIC). Bayesian phylogenetic analysis was performed using MrBayes (Ronquist et al., 2012) with the Markov Chain Monte Karlo Method (MCMC). MCMC chains were run for 10 million generations and obtained a standard deviation of split frequency less than 0.01. The ML phylogenetic tree was inferred using RaxML (Stamatakis 2014) and a consensus tree was obtained with 1000 bootstrap replicates using default parameters. The final phylogenetic tree was displayed, annotated, and embellished in FigTree software (http://tree.bio.ed.ac.uk/software/figtree/) (Rambaut 2016).

Table 3.3 Name and GenBank accession numbers of species used in the study.

| Sl.No. | Species | Accession No. |
|---|---|---|
| 1 | *Decapterus russelli* | MN_711693 |
| 2 | *Alectis ciliaris* | NC_025566 |
| 3 | *Alectis indica* | KP_710215 |
| 4 | *Alepes djedaba* | KP_408222 |
| 5 | *Alepes kleinii* | NC_023524 |
| 6 | *Atule mate* | NC_026222 |
| 7 | *Carangoides armatus* | NC_004405 |
| 8 | *Carangoides bajad* | LC_557137 |
| 9 | *Carangoides equula* | NC_025644 |
| 10 | *Carangoides malabaricus* | NC_023968 |
| 11 | *Carangoides plagiotaenia* | MT_677872 |
| 12 | *Carannx ignobilis* | NC_022932 |
| 13 | *Caranx melampygus* | AP_004445 |
| 14 | *Caranx tille* | NC_029421 |
| 15 | *Decapterus macarellus* | NC_026718 |
| 16 | *Decapterus macrosoma* | KF_841444 |
| 17 | *Decapterus maruadsi* | NC_024556 |
| 18 | *Decapterus tabl* | MN_102718 |
| 19 | *Elagatis bipinnulata* | NC_029880 |
| 20 | *Gnathandon speciosus* | NC_054367 |
| 21 | *Megalaspis cordyla* | NC_025565 |
| 22 | *Parastromateus niger* | KJ_102332 |
| 23 | *Pseudocaranx dentex* | MZ_359280 |

| | | |
|---|---|---|
| 24 | *Selar crumenophthalmus* | NC_023954 |
| 25 | *Selaroides leptolepis* | NC_029184 |
| 26 | *Seriola dumerili* | AB_517558 |
| 27 | *Seriola lalandi* | AB_517557 |
| 28 | *Seriola quinqueradiata* | AB_517556 |
| 29 | *Seriola rivoliana* | NC_027183 |
| 30 | *Seriolina nigrofasciata* | NC_028420 |
| 31 | *Trachinotus blochii* | NC_024026 |
| 32 | *Trachinotus carolinus* | NC_024184 |
| 33 | *Trachinotus ovatus* | KJ_642220 |
| 34 | *Trachurus japonicus* | AP_003092 |
| 35 | *Trachurus trachurus* | AB_108498 |
| 36 | *Uraspis helvola* | NC_033402 |
| 37 | *Uraspis secunda* | NC_029488 |

### 3.3.5 Divergence time estimation

The estimation of divergence times of 37 species of Carangidae was carried out with RelTimeML method in MEGAX using GTR+G+I modeling (Mello 2018). To avoid the inconsistency between divergence time and phylogenetic analysis, the tree obtained from Bayesian inference was imported to MEGA X for the estimation of divergence time. The time tree web resource (http://www.timetree.org/) (Kumar et al., 2017) was used to derive calibration boundaries (minimum or/and maximum) and 32 calibration constrains were used to construct the time tree. The same database was used to establish the time tree of 27 genera of Carangidae.

## 3.4 Results

### 3.4.1 Mitochondrial genome organization and composition

The assembled *D. russelli* mitochondrial genome consisted of 16542 bp. It has been determined, characterized and deposited in the GenBank under the accession number MN711693. The organization of the genome is congruent with that described for other teleost fishes. It included a standard set of 13 putative PCGs, 22 tRNA genes, 2 rRNA genes, and a control region (D loop) (Figure 3.1). Of these, 28 genes were encoded on the heavy strand (H) and ND6 and 8 tRNA genes (tRNA –Gln, tRNA-Ala, tRNA-Asn, tRNA- Cys, tRNA- Tyr, tRNA-Ser (TGA), tRNA-Glu and tRNA- Pro), which were encoded on the light strand (L). The characteristic constituents of *D.*

*russelli* mitochondrial genome were depicted in Table 3.4. The total length of protein coding gene (PGCs) was 11425 bp. The 22 tRNA genes are varying between 67-72 bp and interspersed throughout the genome. The 12S rRNA and 16S rRNA genes were 954 and 1723 bp in length respectively and are observed between tRNA-Phe and tRNA-Leu (TAA) separated by tRNA-Val. It was discerned that there are 7 regions of gene overlapping ranging from 1-7 bp and 11 spacer regions varying in length from 1-38 bp with the longest intergenic spacer region observed between tRNA- Asn and tRNA- Cys. The overlaps are detected both among tRNA (tRNA-Gln-tRNA-Met; tRNA-Thr-tRNA-Pro) and protein coding gens (*ATP8-ATP6*, *ATP6-COXIII*, *ND5-ND6*), but hardly observed between tRNA and PCGs. The control region is 840 bp in length and located between tRNA-Pro and tRNA-Phe as observed in most other vertebrate mitochondrial genome.

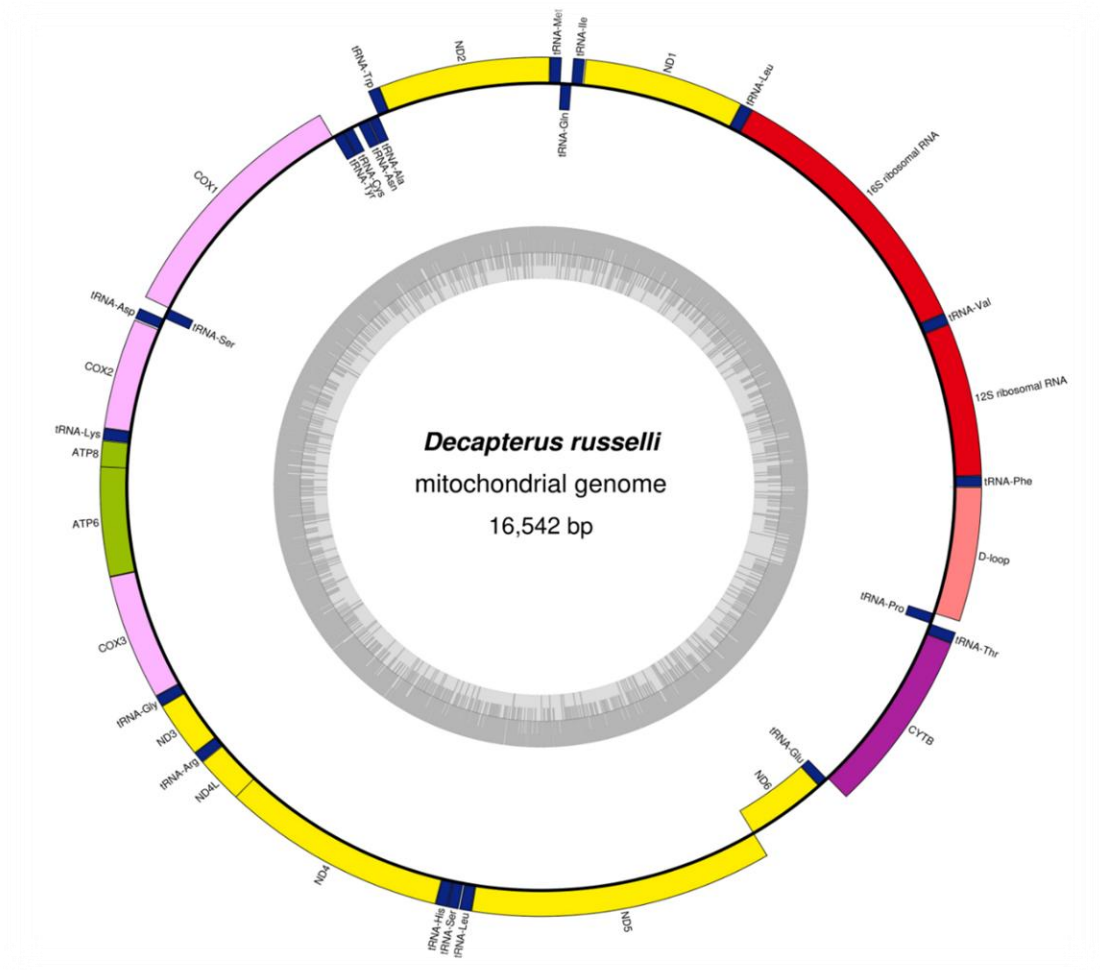


Figure 3.1 Gene map of the complete mitochondrial genome for *D. russelli*

Table 3.4 Sequence characteristics of annotated mitochondrial genes of *D. russelli*. For adjacent genes positive number, negative number and zero indicated spacer, overlap and continuous respectively.

| Locus | Strand | Position | Size (bp) | Codon | | AT Skew | GC Skew | Anticodon | Intergenic nucleotide |
|---|---|---|---|---|---|---|---|---|---|
| tRNA-Phe | H | 1-68 | 68 | Start | Stop | 0.297 | 0.030 | GAA | |
| 12SrRNA | H | 69-1022 | 954 | | | 0.180 | -0.079 | | |
| tRNA-Val | H | 1023-1094 | 72 | | | 0.224 | -0.056 | TAC | |
| 16SrRNA | H | 1095-2817 | 1723 | | | 0.191 | -0.106 | | |
| tRNA-Leu(TAA) | H | 2818-2891 | 74 | | | 0.105 | -0.060 | TAA | |
| ND1 | H | 2892-3866 | 975 | ATG | TAA | -0.060 | -0.363 | | 5 |
| tRNA-Ile | H | 3872-3941 | 70 | | | 0.117 | 0.110 | GAT | |
| tRNA-Gln | L | 3941-4011 | 71 | | | 0.161 | -0.210 | TTG | -1 |
| tRNA-Met | H | 4011-4080 | 70 | | | 0.00 | -0.057 | CAT | |
| ND2 | H | 4081-5125 | 1045 | ATG | T-- | 0.060 | -0.500 | | |
| tRNA-Trp | H | 5126-5196 | 71 | | | 0.315 | 0.00 | TCA | 1 |
| tRNA-Ala | L | 5198-5266 | 69 | | | 0.171 | -0.285 | TGC | 1 |
| tRNA-Asn | L | 5268-5340 | 73 | | | 0.232 | -0.236 | GTT | 38 |
| tRNA-Cys | L | 5379-5445 | 67 | | | 0.00 | -0.032 | GCA | |
| tRNA-Tyr | L | 5446-5515 | 70 | | | 0.134 | -0.211 | GTA | 1 |
| COI | H | 5517-7067 | 1551 | GTG | TAA | -0.095 | -0.187 | | |
| tRNA-Ser(TGA) | L | 7068-7138 | 71 | | | 0.082 | -0.180 | TGA | 3 |
| tRNA-Asp | H | 7142-7212 | 71 | | | 0.026 | -0.002 | GTC | 7 |
| COII | H | 7220-7910 | 691 | ATG | T-- | 0.049 | -0.289 | | |
| tRNA-Lys | H | 7911-7984 | 74 | | | -3.614 | -0.141 | TTT | 1 |
| ATP8 | H | 7986-8150 | 165 | ATG | TAG | -0.053 | -0.430 | | -7 |
| ATP6 | H | 8144-8827 | 684 | ATG | TAA | -0.053 | -0.430 | | -1 |
| COIII | H | 8827-9611 | 785 | ATG | TA- | -0.053 | -0.280 | | |
| tRNA-Gly | H | 9612-9681 | 70 | | | 0.129 | -0.084 | TCC | |
| ND3 | H | 9682-10030 | 349 | ATG | T-- | -0.138 | -0.363 | | |
| tRNA-Arg | H | 10031-10099 | 69 | | | -0.021 | 0.083 | TCG | 1 |
| ND4L | H | 10101-10397 | 297 | ATG | TAA | -0.113 | -0.380 | | |
| ND4 | H | 10391-11771 | 1381 | ATG | T-- | -0.003 | -0.396 | | |
| tRNA-His | H | 11772-11843 | 72 | | | 0.00 | -0.067 | GTG | |
| tRNA-Ser(GCT) | H | 11844-11911 | 68 | | | 0.173 | -0.128 | GCT | |
| tRNA-Leu(TAG) | H | 11917-11989 | 73 | | | 0.149 | -0.0930 | TAG | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ND5 | H | 11990-13828 | 1839 | ATG | TAG | 0.003 | -0.394 | | -4 |
| ND6 | L | 13825-14346 | 522 | ATG | TAG | -0.445 | 0.327 | | |
| tRNA-Glu | L | 14347-14415 | 69 | | | 0.210 | -0.300 | TCC | 4 |
| Cyt b | H | 14420-15560 | 1141 | ATG | T-- | -0.070 | -0.352 | | |
| tRNA-Thr | H | 15561-15632 | 72 | | | -0.030 | -0.025 | TGT | -1 |
| tRNA-Pro | L | 15632-15702 | 71 | | | 0.134 | -0.378 | TGG | |
| D loop | H | 15703-16542 | 840 | | | 0.037 | -0.104 | | |

Comparative analysis among Carangidae revealed the overall mitogenome length of the family varied from 16530 bp (*S. rivoliana* and *S. dumerili*) to 16610 bp (*S. crumenophthhalmus*). The overall length of PCGs varied from 11416 bp (*G. speciosus*) to 11433 bp (*Seriola* Spp.), rRNA varied from 2655 bp (*G. speciosus*) to 2683 bp (*A. kleinii*), tRNA varied from 1550 bp (*P. dentex*) to 1564 bp (*T. blochii*), and the control region was varied from 841 bp (*S. rivoliana)* to 904 bp (*S. crumenophthalmus*) among other carangid species (Figure 3.2).

### 3.4.2 Nucleotide Composition

The overall H strand nucleotide composition of *D. russelli* mitochondrial genome was 27.5% A, 30.2% C, 25.4% T and 16.9% G with an A+T content of 52.9%. Of these, the control region of *D. russelli* possessed highest percentage of Adenine and Thymine nucleotides (63.6%), whereas the A+T content of overall PCGs (51.7%) was determined to be the lowest. The A+T content of rRNA and tRNA genes were determined to be 53.7% and 54.2% respectively. The AT skew and GC skew of *D. russelli* whole mitogenome was determined to be 0.0396 and -0.02823 respectively. Both AT and GC skew values for overall protein coding genes were determined to be negative, whereas the situation was different when we analyzed the base skew values with reference to the individual PCGs. The GC skew values of all PCGs were negative except the case with the L strand encoded gene, *ND6* which was greater than zero.

The comparative analysis among Carangidae have shown that, the GC and AT skew values of PCGs were found to be negative except AT skew values for *M. cordyla and C. tille* which were found positive. A clear anti-G bias was detected at the third codon position of all carangids. The base skew analysis of 22 concatenated tRNA genes revealed a positive AT skew (0.125) and a negative GC skew (-0.098) which was analogous with other sequenced Carangidae species. The

comparative result showed that AT skew varied from 0.1058 (*S. nigrofasciata*) to 0.224 (*T. ovatus*)

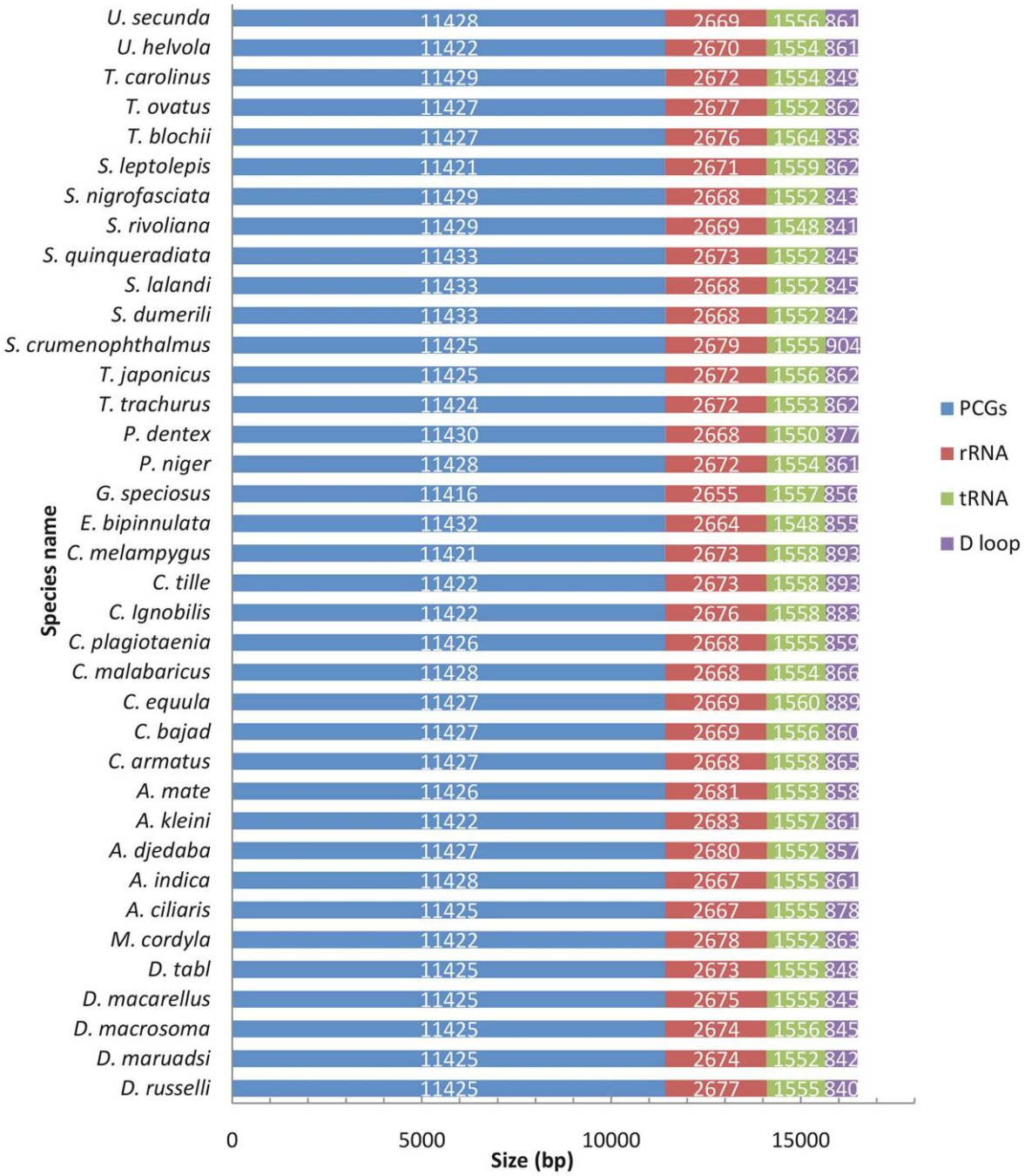

Figure 3.2 Length (bp) of PCGs, rRNA, tRNA and control region of 37 species of Carangidae.

and GC skew varied from -0.1205 (*S. rivoliana*) to -0.091 (*T. carolinus*). The same trend was also observed in the case of rRNA genes that it also revealed a positive AT skew (0.188) and a negative GC skew (-0.103). The comparative analysis among Carangidae revealed that the AT and GC skew

of rRNA varied from (0.1615) to (0.2256) and (-0.1247) to (-0.0772) respectively. Further, the AT skew value was positive (0.038) and GC skew value was negative (- 0.104) in the control region of *D. russelli*. Significant variation was observed in the AT and GC skew values of other Carangidae which was ranged from -0.047 (*S. crumenophthalmus*) to 0.065 (*T. blochii*) and -0.278 (*C. ignobilis*) to -0.093 (*D. maruadsi*) respectively.

### 3.4.3 Protein coding genes

The total length of protein coding gene was 11425 bp, ranged in size from 165 bp (ATP8) to 1839 bp (ND5) accounting for 69% of the entire genome. The 13 PCGs in *D. russelli* mitogenome was similar to other vertebrates consisting of 3 cytochrome oxidase complex (*COX1-III*), 7 NADH oxido reductase complex (*ND1-ND6, ND4L*), a cytochrome oxidoreductase complex (*Cyt b*) and 2 ATP synthases (*ATP6* and *ATP8*). All the PCGs are located on the H strand except *ND6* which was expressed on the light strand (Figure 3.1). Among the 13 PCGs, 12 of them sharing the same orthodox initiation codon, ATG whereas, *COX 1* has a quiet different initiation codon and starts with GTGinstead of ATG (Table 3.4). Besides, six genes exhibited incomplete termination codons, either TA (*COIII*) or T (*ND2, COII, ND3, ND4*, and *Cyt b*). Remaining genes revealed complete stop codons in open reading frames either TAA (*ND1, COI, ATP6* and *ND4L*) or TAG (*ATP8, ND5* and *ND6*). The comparative analysis among Carangidae revealed that most of the PCGs were started with ATN initiation codon. *COX1* of all species start with GTG except ATC in *G. speciosus*. All the other PCGs start with ATG except ATA for *ATP 6* (*A. djedaba*, *A. kleinii*, *A. mate, C. ignobilis*, *C. melampygus*, *C. equula*, *C. tille and M. cordyla)* and *ND4L* (*T. trachurus*), and GTG for *ND5* (*T. blochii, T. carolinus* and *T. ovatus*).

A total of 3800 amino acids of PCGs are encoded in *D. russelli* mitogenome. The codon usage patterns are shown in Figure 3.3 a. The amino acid Leu was represented by six different codons and the rest of them by either four or two. The amino acids Leu (16.46), Ala (8.75), and Thr (7.96) were most frequent in *D. russelli* mitogenome. Among these, the frequency of Leu (CTC, CTA), Ala (GCC), and Thr (ACC) were the highest. In addition, the frequencies of stop (TAG), Cys (TGT) and Arg (CGG) in PCGs were the lowest. However, relative synonymous codon usage (RSCU) analysis indicated that the codons Ser (UCC), Leu (CUC and CUA), and Ala (GCC) were most frequently presented while Leu (UUG), Ser (AGU) and Ala (GCG) were rare (Figure 3.3 b).

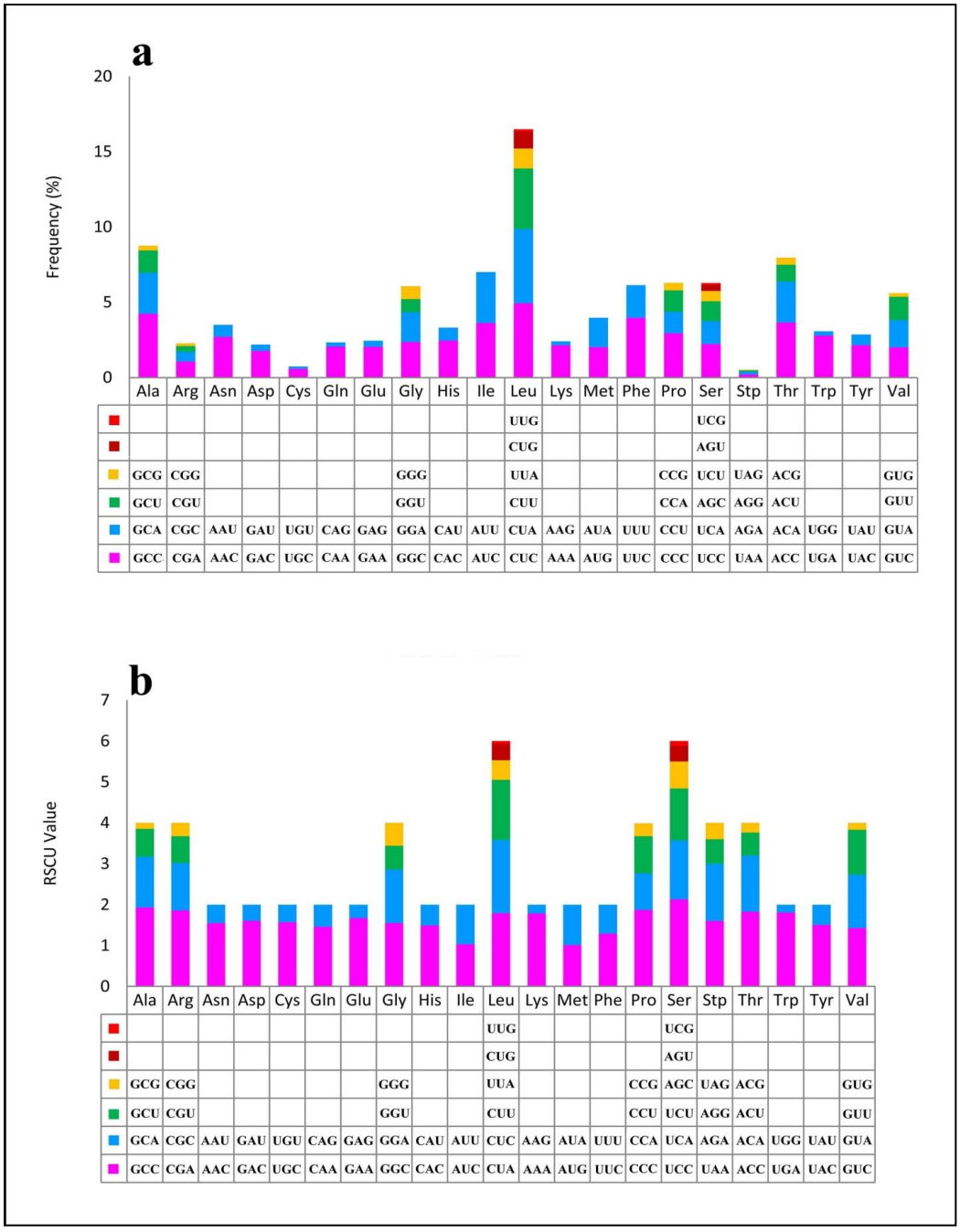
a
Frequency (%)
20
15
10
5
0
Ala Arg Asn Asp Cys Gln Glu Gly His Ile Leu Lys Met Phe Pro Ser Stp Thr Trp Tyr Val
UUG UCG
CUG AGU
GCG CGG GGG UUA CCG UCU UAG ACG GUG
GCU CGU GGU CUU CCA AGC AGG ACU GUU
GCA CGC AAU GAU UGU CAG GAG GGA CAU AUU CUA AAG AUA UUU CCU UCA AGA ACA UGG UAU GUA
GCC CGA AAC GAC UGC CAA GAA GGC CAC AUC CUC AAA AUG UUC CCC UCC UAA ACC UGA UAC GUC
b
RSCU Value
7
6
5
4
3
2
1
0
Ala Arg Asn Asp Cys Gln Glu Gly His Ile Leu Lys Met Phe Pro Ser Stp Thr Trp Tyr Val
UUG UCG
CUG AGU
GCG CGG GGG UUA CCG AGC UAG ACG GUG
GCU CGU GGU CUU CCU UCU AGG ACU GUU
GCA CGC AAU GAU UGU CAG GAG GGA CAU AUU CUC AAG AUA UUU CCA UCA AGA ACA UGG UAU GUA
GCC CGA AAC GAC UGC CAA GAA GGC CAC AUC CUA AAA AUG UUC CCC UCC UAA ACC UGA UAC GUC

Figure 3.3 (a) Codon frequency (b) RSCU (Relative Synonymous Codon Usage) of mitochondrial genome of *D. russelli.*

Analysis of the rate of non synonymous substitution (Ka) to the rate of synonymous substitution (Ks) of PCGs revealed a smaller value for Ka than Ks in all 13 PCGs. Besides, the average Ka/Ks ratios substantially differed among PCGs and varied from 0.008 in *COI* to 0.469 in *ATP8*. We

estimated the degree of mitochondrial gene conservation by assessing the total p-genetic distance between 37 Carangidae species. In our analysis, we obtained the highest overall p genetic distance for the *ATP8* gene (0.301) and the lowest value for the *COI* gene (0.019) based on the data of the first and second nucleotide position of codons and the full-length comparison of each gene. Based on these results it could be inferred that gene, *ATP8* has the fastest evolutionary rate among Carangidae and *COI* the slowest. All the genes had overall high p-genetic distance values for wobble nucleotide position, ranging from 0.393 to 0. 564.This result was consistent with previous reports in other fish species that most of the differences occurred at the 3rd codon position of PCGs in mtDNA (Zhuang et al., 2013).

### 3.4.4 Transfer and ribosomal RNA genes

*D. russelli* mitogenome contained 22 tRNA genes which was typical of metazoan mitogenome. Altogether the tRNA genes contributed 1555 bp to the entire genome ranging from 67 bp (tRNA-Cys) to 74 bp (tRNA-Lys (TAA)). Fourteen tRNAs were encoded on the H strand while remains were located on the L strand (Table 3.4). The secondary structure of tRNA genes predicted with ARWEN (Laslett and Canbäck 2008) make it apparent that all of the tRNAs could be folded into a canonical cloverleaf structure in *D. russelli* except for tRNA-Ser (GCT) which was not found to have a recognizable DHU stem. The comparative analysis with other Carangidae species depicted the total length of tRNAs varied from 1548 bp (*S. rivoliana* and *E. bipinnulata)* to 1564 bp (*T. blochii*) (Figure 3.2). The comparative analysis showed that anticodons of these 22 tRNA genes were identical to those reported in other Carangidae species. Although there existed a general one to one correspondence of codon –anticodon, tRNA genes for Serine (TGA and GCT) and Leucine (TAA and TAG) were determined by two types of anticodons. Furthermore, G-U mismatches were observed for 16 of the tRNAs in their respective secondary structures. Five of the tRNA genes were appeared to have A-C mismatches (tRNA-Val (TAC), tRNA-Leu (TAA), tRNA-Ile (GAT), tRNA-Arg (TCG), tRNA-His (GTG) in their respective amino acid accepter stems. Similarly, two A-A mismatches, each for tRNA-Phe (GAA) and tRNA-Ser (GCT) and two C-C mismatches each for tRNA-Leu (TAG) and tRNA-Thr (TGT) were also detected. Besides, mismatched base pairings were observed on comparative structural analysis in all 22 tRNAs with highest in tRNA Ser (GCT) and tRNA-Val and lowest in tRNA-Lys (Figure 3.4).

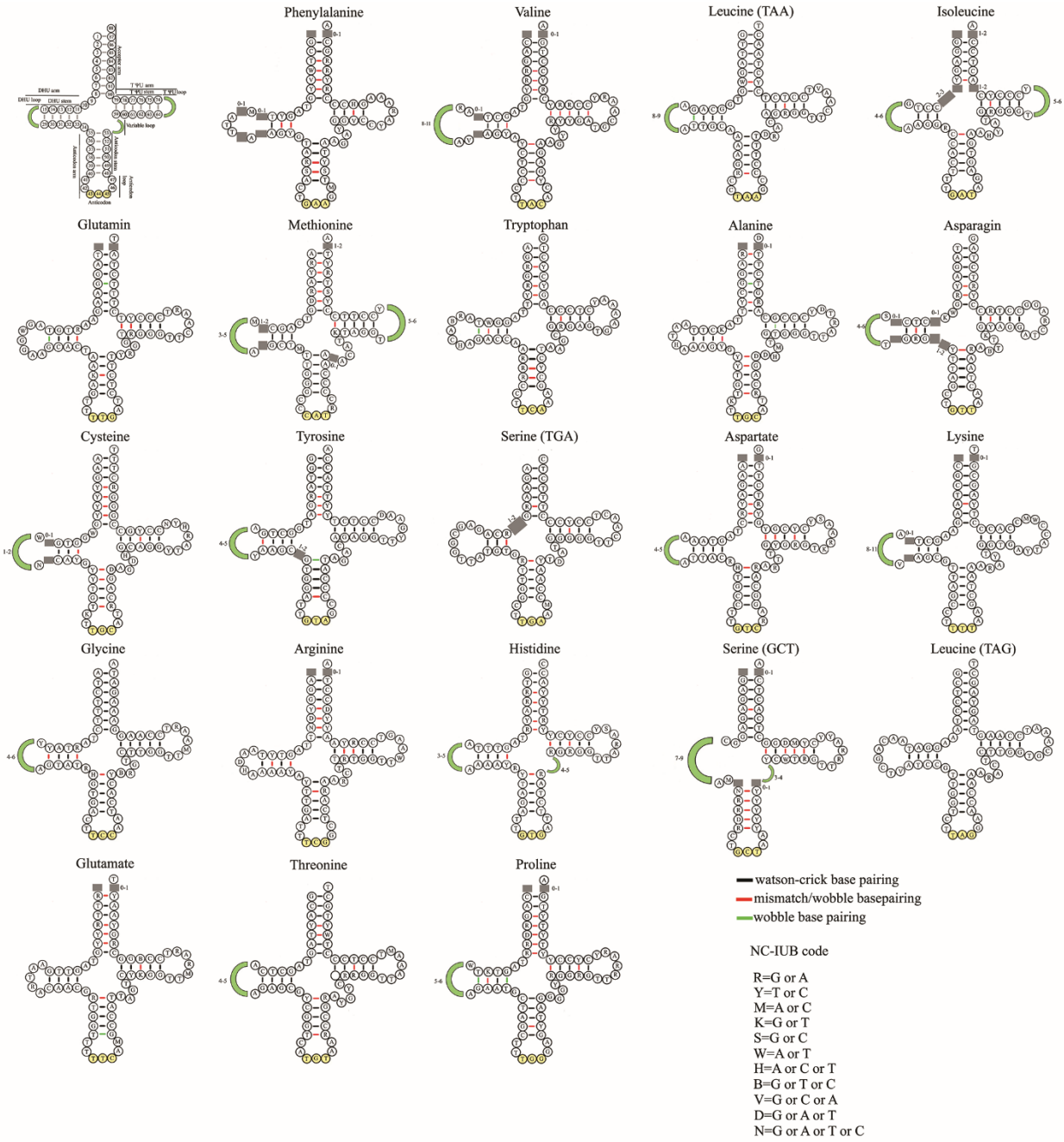


Figure 3.4 Secondary structure of tRNAs showing structural variations in 37 species. The first structure represents the nucleotide positions common to all tRNAs (1-88) and details of stem- loop. Length variations in stems and loops are indicated by grey squares and green arcs respectively.

In addition, the tRNA genes of all Carangidae showed variation with regard to the stem and loop length (bp) and the frequency of occurrence of wobble base pairs. We also detected a tridecameric mTERF (mitochondrial transcription termination factor) binding site (Figure 3.5) in the tRNA-Leu (TAA) gene.

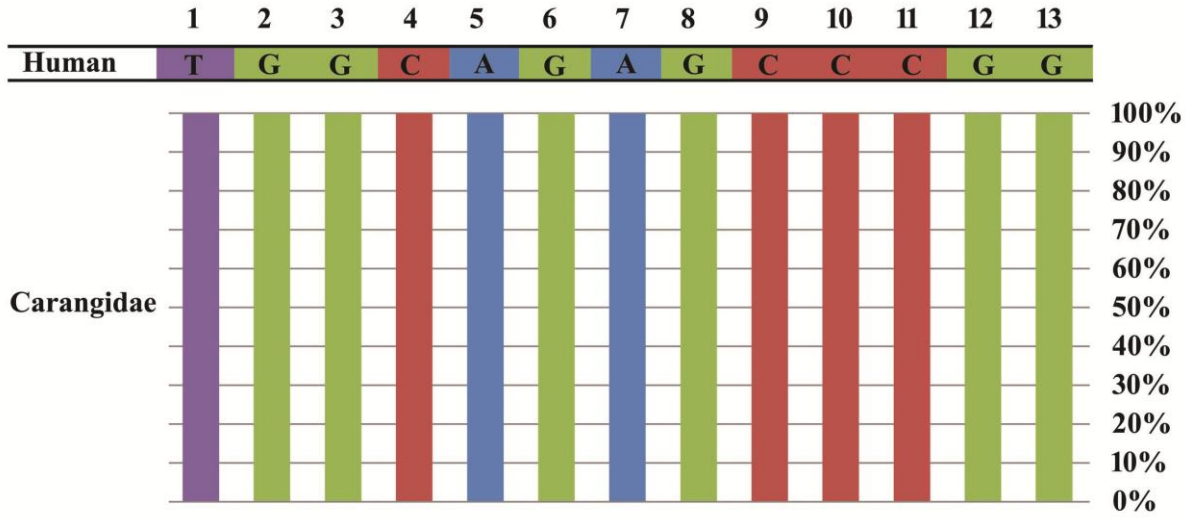


Figure 3.5 Base frequencies (%) of the mitochondrial transcription termination factor binding site in the tRNA-Leu (TAA) gene in the mitogenomes of 37 Carangids.

Two rRNA genes, a small (12srRNA) and large (16sRNA) subunit were located in the H strand of *D. russelli*. The total length of two concatenated rRNA genes was estimated to be 2677 bp with an A+T content of 53.7%. The 12s rRNA and 16sRNA were 954 bp and 1723 bp in length respectively. Consistent with the results from other mitochondrial genomes described so far, these two rRNA genes were positioned between tRNA-Phe and tRNA-Leu (TAA) separated by tRNA-Val (Jang et al., 2015; Li et al., 2016; Xiao et al., 2016). The AT content of 12S rRNA gene was 52.2% with a positive AT skew of 0.172 and negative GC skew of -0.079. Similarly, the AT content of 16S rRNA gene was 54.4% with a positive AT skew (0.191) and negative GC skew (-0.116). Moreover, the presence of a higher proportion of A and C bases in rRNA genes indicate an AC rich trend which is in line with previous reports from other bony fishes (Zardoya and Meyer 1997; Shi et al., 2016; Zhu et al., 2017). The comparative analysis with other Carangidae species (Figure 3.2) showed that, the length of two rRNA genes varied from 2655 bp (*G. speciosus*) to 2683 bp (*A. kleinii*).

### 3.4.5 Control region

The control region of *D. russelli* was shorter than other carangids, consisting of 840 bp flanked by tRNA-Pro and tRNA-Phe on either side (Figure 3.1). The control region comprised of highest A+T percentage (63.6%) compared with other regions of mitogenome. This result obtained here are in exceptionally good agreement with existing findings (Prabhu et al., 2020; Zhang et al., 2022) and consistent with the result from other Carangidae species. In addition, the length of control region was varied from 841 bp (*S. rivoliana)* to 904 bp (*S. crumenophthalmus*) among other Carangidae species (Figure 3.2).

Consistent with the recognition sites in many marine fish (Lee et al., 1995), we have detected the 3 central conserved sequence blocks (CSB- F, CSB- E, and CSB-D) and 3 conserved sequence blocks (CSB- 1, CSB-2, and CSB- 3) in all taxa. Palindromic sequence motifs - 'TACAT' and 'ATGTA' were found in multiple copies distributed towards the 5' end of the control region (Figure 3.6) and are believed to act as the termination site of heavy strand replication (Zhu et al., 2008). No tandem repeat sequences were detected in the control region of Carangidae, but the T homopolymer of 5-12 nucleotides between CSB-D and CSB-1 was found in all taxa. Along with these, discontinuous multiple interval repeats of ATATTA and TATAAT were also observed in the control region of Carangidae.

### 3.4.6 CG View comparison tool (map)

We compared the complete mitochondrial genome of *D. russelli* with other available mitogenomes of the family Carangidae. A visual map was obtained illustrating the similarity of both nucleotide and coding DNA sequences of the mitogenome of Carangidae with *D. russelli* as the reference sequence. The coding DNA sequences showed high percentage of identity between the species than the nucleotide sequences of mitogenome. The control region contributed greater percentage of differences among nucleotide sequences. A total of 12 COGs (Clusters of orthologous groups) of proteins were detected from11 PCGs.

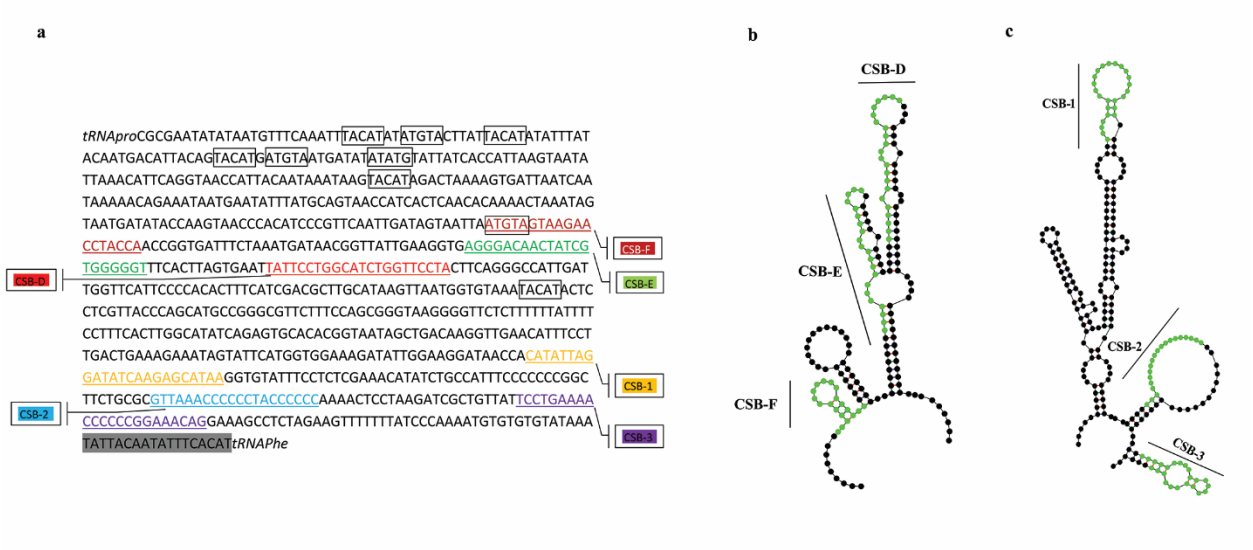


Figure 3.6 Sequence and structure of the mitochondrial DNA control region of carangids (a) Features of the control region. CSB-F, -D, -E: central conserved sequence blocks; CSB-1, -2, -3: conserved sequence blocks. Palindromic sequence motifs 'TACTA' and 'ATGTA' are framed. Grey colored bar represents the characteristic 3' terminal sequence. (b) The secondary structure identified in CSB-F, CSB-D, and CSB -E. (c) Secondary structure identified in CSB-1, CSB-2, and CSB-3.

### 3.4.7 Phylogenetic analysis

The phylogenetic position of *D. russelli* is shown in Figure 3.7 relative to 36 species of carangids based on 1380 bp sequences. Analogous topologies were obtained for both maximum likelihood analysis (ML) and Bayesian inference (BI) with almost 100% bootstrap support and strong posterior probability values, respectively. The result implied that 37 species formed 3 lineages corresponding to the subfamilies Caranginae, Naucratinae, and Trachinotinae. The reconstructed phylogenetic tree places *D. russelli* in the same branch with other *Decapterus* spp. and forms a sister group to the genus *Trachurus* and the findings support the previous classification of carangids ( Zhu et al., 2008; Li et al., 2020).

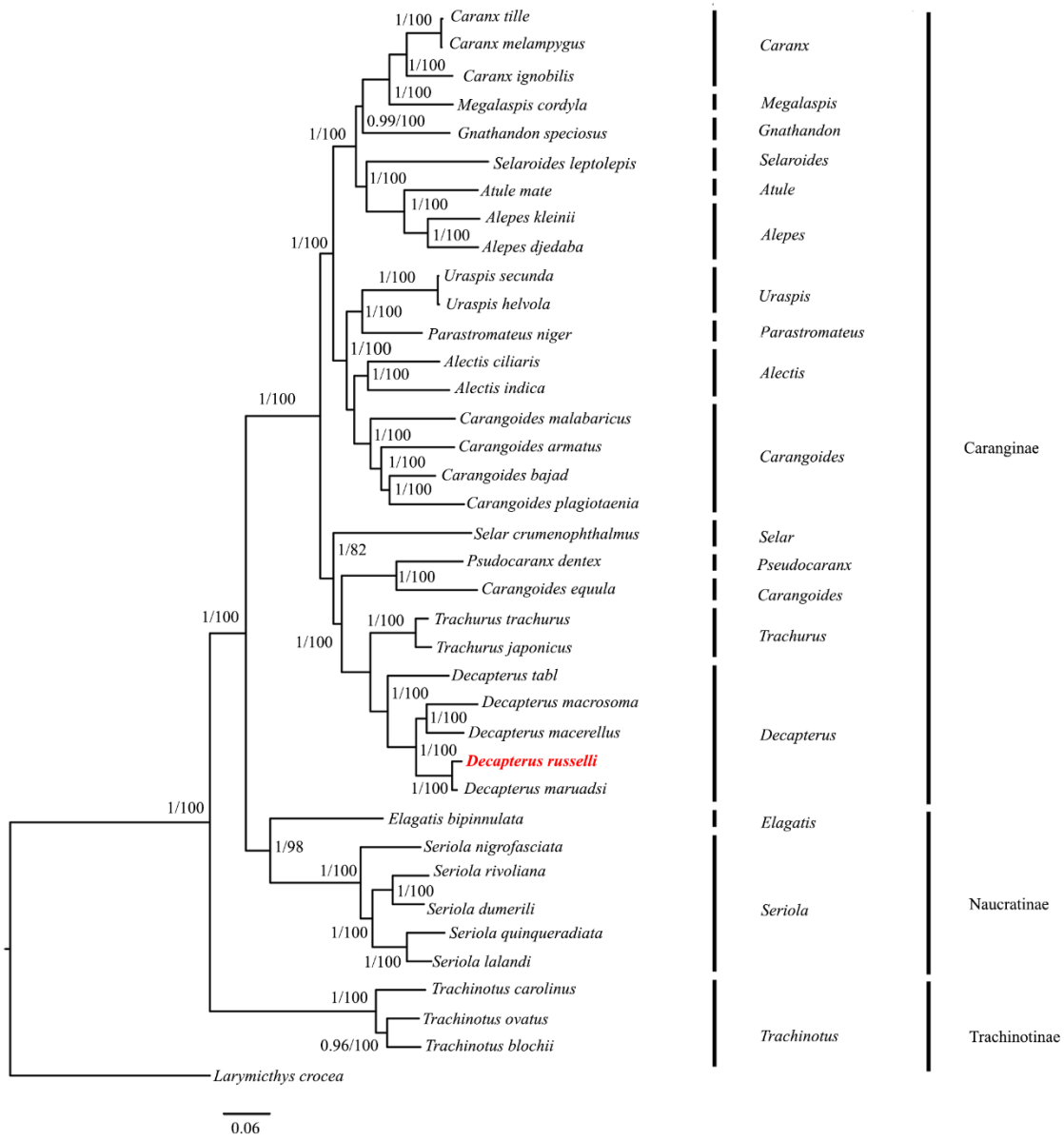


Figure 3.7 Phylogenetic tree inferred from the nucleotide sequences of 13 concatenated PCGs of 37 species of Carangidae. Bayesian posterior probabilities (left) and bootstrap support values (right) are superimposed with each node.

### 3.4.8 Divergence time estimation

The RelTime ML analysis revealed that the differentiation of *D. russelli* could be dated back to about 4.69 Mya in the early Pliocene (Figure 3.8). The family Carangidae had diverged from other lineages when the Cretaceous–Paleogene extinction event occurred and it was estimated to be around 79 Mya towards the end of the cretaceous.

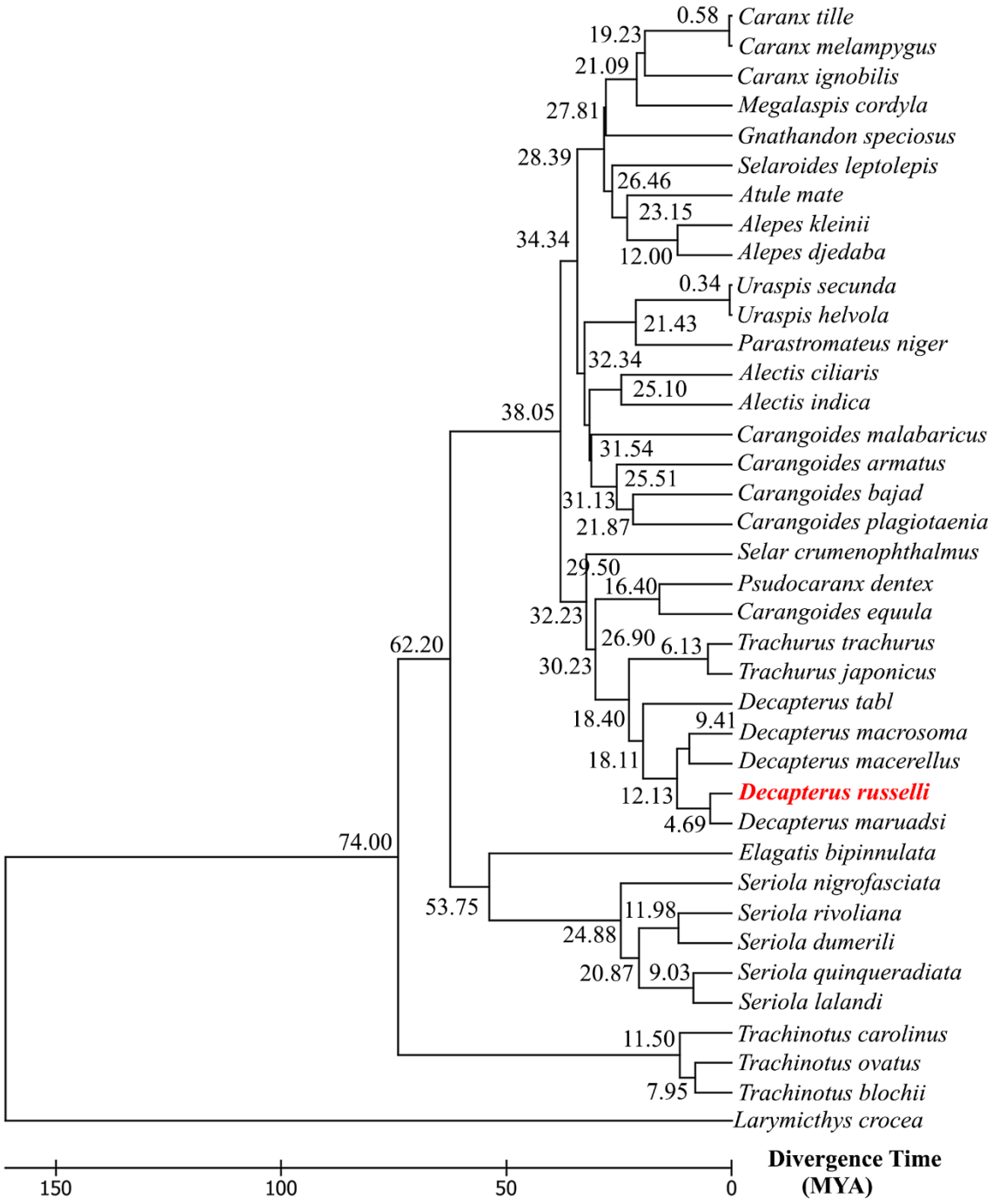


Figure 3.8. Timetree chronogram of 37 species of Carangidae for the 13 PCGs inferred from Bayesian topology. Divergence times were estimated using 32 calibrations.

## 3.5 Discussion

The comparative analysis revealed that the overall length of PCGs was almost identical across the family and that of *D. russelli* was exactly same with their respective counterparts in other *Decapterus* species. Similar result was also obtained with tRNA and rRNA genes, which were

varied by a maximum of 16 bp and 19 bp respectively between species. It was discerned that, length diversification was observed to be maximum with respect to the control region ranging from 840 bp (*D. russelli*) to 904 bp (*S. crumenophthalmus*) in the Carangidae examined. Among the species, *S. crumenophthalmus* possessed the longest mitogenome and *S. rivoliana* and *S. dumerili* were found to have the shortest mitogenome. It was apparent that both species do not exhibit remarkable variation with respect to their PCGs, tRNA, and rRNA, thus the variation of 61-62 bp in the control region alone contributed to the difference in their whole mitogenome length. It is therefore speculated that, the variation in the length of control region might be responsible for the differences in whole mitogenome length. This may be due to the control region especially majority of its primary sequences do not appear to be involved in the regulatory function as this region shows much variation even among closely related species (Broughton et al., 2001)

The A+T content of *D. russelli* mitogenome indicate significant strand specific bias which is in line with the result observed in most vertebrates (Yang et al., 2018). Furthermore, the positive AT skew and negative GC skew values suggests that the A and C are predominant base constituents of H strand than T and G with overall nucleotide composition was strictly C skewed. The bias against G was clearly presented in accordance with those results reported previously in other fishes (Norfatimah et al., 2014) and teleosts species (Xie et al., 2016). In addition, the 12 of the 13 PCGs of *D. russelli* shows a clear preference for C in the codon wobble position, conversely *ND6*, the only gene encoded by the L strand was G skewed. These results are consistent with the previous observations (Yang et al., 2018) and suggest a remarkable anti-G bias at third codon position of PGCs. There was a higher proportion of pyrimidines (C+T= 66.6%) in the $2^{nd}$ codon position compared to purines which is believed to be responsible for the hydrophobic characteristics of proteins (Yang et al., 2018).

On indexing the evolutionary rate of PCGs, we observed the value of Ka was significantly smaller than that of Ks in all 13 PCGs, suggesting that there is a much lesser chance for a mutation to be different between species that can modify the amino acid sequences of a protein sufficiently to change its function, than one which is silent (Hurst 2002). The fundamental features of genome evolution were largely determined by the influence of two non-adaptive forces, mutation pressure and genetic drift (Shi et al., 2016). Nevertheless, the deleterious effect of mutation is very difficult to establish under purifying selection (Yang et al., 2018). In all 13 PCGs, the average Ka/Ks ratio varied from 0.0089 in *CO1* to 0.469 in *ATP8*. Altogether the

results indicated that the functional genes of these carangids undergone strong purifying selection to eliminate deleterious mutations and thereby keeping the proteins as such.

The comparative analysis of 37 Carangidae revealed that most of the tRNAs are structurally different in both stem and loop. However, 2 tRNA genes, tRNA-Trp and tRNA-Leu (TAG) exhibited similarity in their secondary structure among Carangidae. In addition, length variation was detected in all four stems. The AC stem of all the tRNAs was 5 bp in length except for tRNA-Ser (GCT), which displayed length variation (5 bp or 6 bp). Although the TΨU stem of most tRNAs was 5 bp in length, tRNA-Phe (4 bp), tRNA-Met (4 or 5 bp), tRNA-Lys (4 bp) and tRNA-Ser (GCT) (6 bp) displayed length variation. With regard to the accepter stem (AA), whereas tRNA-Ser (TGA) displayed a length of 6 bp, rest of the tRNAs rather displayed length variation of either 7 bp or a range of 7 to 8 bp. Even though the DHU stem exhibited a length mostly of 4 bp with a range of 3bp to 5 bp, the tRNA-Ser (GCT) of all the examined Carangidae had only a small loop without making a stem. Regarding the four loops, the length of anticodon loop was fixed (4 nucleotides), whereas the remaining three loops showed considerable variation in length. Of these DHU loop was extremely variable with a range of 3-13 nucleotides. Conversely, variable loop (V loop), mostly 4 nucleotides with range of 3-5 and TΨU loop, mostly 7 nucleotides with a range of 6-9 displayed least variation.

The wobble base pair is a unique feature of RNA structure and their frequently high evolutionary conservation (Sprinzl et al., 1998) underscores its functional and structural significance. Due to its thermodynamic stability the G-U often substitute G-C or A-T base pairs and it is differed from Watson-Crick and other mismatched base pairs owing to its structural, conformational, and functional properties (Varani and McClain 2000). Diverse proteins and other ligands specifically target tRNA exploit these features (Satoh et al., 2016) and thus the comparative analysis of tRNAs are very crucial for understanding the structural and functional features of mitogenomes. A total of 25 base pair sites were detected in 37 Carangide species (8 in AA stem+5 in DHU stem+6 in AC stem+6 in TΨU stem). Seven sites from each stem (1-87, 2-86 and 8-80 base pair sites in the AA stem, 12-32 and 14-30 in the DHU stem, 40-48 in the AC stem, and 64-74 in the TΨU stem) revealed a high rate of (100%) Watson-Crick and Wobble base pairing (hydrogen bonding) and these sites may play a pivotal role in maintaining stem structure.

An important tridecameric sequence (5'TGGCAGAGCCCGG3') reported in the human mitochondrial tRNA-Leu (TAA) gene (Norfatimah et al., 2014; Xie et al., 2016) was also

identified in Carangidae in the same region as the corresponding part of their DHU arm. The mTERF binds to this site with high affinity and regulates the level of transcription from the rRNA genes into the remaining genes of the major coding strand (Nardi et al., 2001). All bases in the 13 nucleotide positions identified in 37 carangid taxa were completely identical to those in humans suggesting that this site in carangids may follow the same mechanism of function as proposed for humans.

Comparative mitogenomics of carangids revealed high variability in the mitochondrial control region due to length variations and the accumulation of base substitutions and indels. We observed exactly the same sequences of CSB-F in *D. russelli* identified in Carangidae mitochondrial control region (Zhu et al., 2008). But a single substitution was observed both in CSB- E and CSB- D sequences. In addition, substitution of 1-7 nucleotides was detected on comparison between the species in CSB- F sequences. All species except *D. maruadsi* displayed substantial differences from the key sequences of CSB- E (Zhu et al., 2008) and all were substitutions ranging from 1 to 9 nucleotides. Furthermore, *M. cordyla*, *P. dentex* and all the three *Caranx* spp. under study lacked a 'GTGGG'- box which is a typical characteristic of CSB- E in teleosts (Lee et al., 1995). It was discerned that the sequences of CSB- D was most conserved among Carangidae that it apparently exhibits high level of similarity between species. 27 of the species under investigation displayed the same key sequences and the difference marked between species was only a single nucleotide substitution. Although the functions of the CSBs remain unclear, CSB- D is highly conserved in fish families and involved in the regulation of H- strand replication and initiation of D loop structure (Clayton 1982) and may have potential roles in mitochondrial metabolism (Lee et al., 1995). Conserved sequence block (CSB-1, CSB-2 and CSB-3) exhibited apparently high level of variation in Carangidae. This result provides compelling evidence for the fastest evolutionary rate of conserved sequence block domain (Guo et al., 2003) in mitochondrial control region. The sequences of CSBs displayed substitution, insertions and deletions between species. These variations imply rapid evolution of the primary structure in the Carangidae control region, which can provide information for understanding their structural and functional relationship. Furthermore, these conserved regions are involved in the formation of secondary structures in the control region that serve as the target sites for proteins or enzymes (Lee et al., 1995). These conserved sequence motifs formed similar secondary structures in Carangidae although their primary sequences are not conserved. Therefore, the data reinforce that although there is sequence divergence in the conserved sequence motif, their structural elements and locations

appeared to be conserved in Carangidae and some selective constraints on these regions acted to maintain their structure and function.

The comparison of whole genome represents an important part of the analysis as they can provide better understanding of the evolutionary relationship of the source organisms and to identify the genomes which are rapidly evolving or novel genome sequences and unusual functional properties of newly sequenced genomes. Comparing a sequence to previously characterized genomes will also provide information about the functional classification and compositional characteristics of coding DNA sequences. In Carangidae, it has been found that *ND1*, *COI*, *COII* and *COIII* are highly conserved among the DNA coding sequences. In addition, *ATP8* was the least conservative of all species, in good agreement with the results obtained from analysis of Ka/Ks and overall mean p genetic distance. With the exception of *ND6* and *ATP8*, other PCGs expressed orthologous group (COG) clusters. *ND5* was determined to have 2 COGs, pCOG, and cCOG, and the remaining 10 PCGs each expressed a single COG each (cCOG). COGs are a group of proteins inferred to be orthologous i.e. they are direct evolutionary counterparts (Koonin 2002). Lineage-specific duplications (orthologous relationships) exist mainly between gene (protein) families rather than between individual proteins (Natale et al., 2000). The identification of COGs associated with specific PCGs could indicate the presence of a specific protein in a specific species, and it is crucial to determine the functional relevance of these proteins in that specific species (Remm et al., 2001). The results indicated that the COGs present in carangids are involved in inorganic ion transport and metabolism (pCOG) and energy production and conservation (cCOG) (Natale et al., 2000). The ND5 gene has been found to perform these two functions, while energy metabolism is the sole function of the remaining 10 PCGs.

The phylogenetic tree recovered the three carangid subfamilies as monophyletic and it also supported the groupings (Trachinotinae + (Naucratinae + Caranginae)). This result confirmed the phylogenetic relationship of carangids described by previous findings based on morphological synapomorphic characters (Gushiken 1988) and partial sequences of mitochondrial genes (Reed et al., 2001; Damerau et al., 2018). Within the Naucratinae, the monotypic *E. bipinnulata* branched individually early from *Seriolina* and *Seriola* spp. After diverging from *Seriolina,* all *Seriola* species formed a monophyletic clade. Furthermore, both phylogenetic trees indicated two well-separated lineages in Caranginae. The first lineage included the genus *Selar, Trachurus,* and *Decapterus.* This lineage also encompassed the

species *C. equula* on the same branch with *P. dentex* in contrast to other *Carangoides* spp. which were grouped into a different branch. This result confirms the findings of previous studies (Li et al., 2021) and thus provides compelling evidence for the assignment of *C. equula* to its previously described genus, *Kaiwarinus*. The second lineage was the most taxonomically diverse group of the family Carangidae delineated in the present study as it consisted of 10 genera divided into two larger groups. The first lineage comprised the genera: *Caranx+Megalaspis+Gnathandon* as well as *Atule+Alepes.* The latter also included the monotypic *S. leptolepis* which individually branched off from this lineage early. The second lineage included *Uraspis+Parastromateus* and *Alectis+Carangoides.* In addition, a paraphyletic relationship between *Caranx+M. cordyla, Alepes+A. mate,* and *Uraspis+P. niger* was also observed.

Based on the evolutionary analysis, it was observed that the separation of the two groups, Trachinotinae and (Caranginae+Naucratinae) occurred at 74 Mya at the end of the Cretaceous. Further, the split between the subfamilies Caranginae and Naucratinae occurred at 62.20 Mya (Early Paleocene). Although the diversification of most species after the Cretaceous mass extinction occurred in the Paleogene (Zinsmeister 1998), several more recent Neogene radiations can also be observed. The most recent split was observed at 0.34 Mya between the two *Uraspis* species. Our analyzes of the diversification of the genus Trachurus (18.40 Mya, early Miocene) were consistent with previous studies based on the data of morphological characters and ecological aspects (Shaboneyev 1981) and *Cyt b* gene sequence analysis (Cárdenas et al., 2005). The split between *Seriola+Seriolina* and *Elagatis* occurred during the early Paleocene, consistent with Swart et al. (2015) but much younger than Le et al. (2020). The radiation of *Seriola* appears to be 20.87 Mya which is in direct agreement with Le et al. (2020) but slightly predates the estimates by Santini and Carnevale (2015).

## 3.6 Conclusion

The present investigation focused on the characterization of the complete mitochondrial genome of the Indian Scad, *D. russelli*, and a comprehensive comparative analysis of Carangidae with special emphasis on the structure and variability of tRNA and control region. The carangid tRNA was structurally different in both the stem and loop, with the exception of tRNA-Trp, and tRNA-Leu (TAG). These structural features resulted in mismatched or wobble base pairs in tRNAs. The high rate of Watson-Crick and Wobble base pairing in the stem

regions of tRNA suggested their role in stem structure maintenance. The classical cloverleaf secondary structure was retained in all tRNAs except tRNA-Ser (GCT). The presence of an mTERF binding site and a very high evolutionary conservation of its sequence suggest the functional significance of these sites. The high rate of sequence divergence in the flanking regions of conserved sequence motifs indicated the absence of typical mitochondrial control region coding constraints. The presence of evolutionarily conserved secondary structures of the conserved sequence motifs despite sequence divergence revealed the effect of selective constraints on their maintenance. The length variations in protein-coding, tRNA, and rRNA genes among Carangidae were negligible.

The phylogeny inferred from 13 gene concatenated data set resolved the monophyletic lineage of each subfamily and robustly supported the grouping (Trachinotinae+(Caranginae+Naucrautinae)) with the caveat that the subfamily Scomberoidinae could not be included as the mitogenome sequences were not available. Further, the speciation of the genus *Decapterus* occurred before the separation of Tethys Sea and *D. russelli* diverged before the mid-Pliocene warm period.

## 3.7 Future research directions

The comparative mitogenomic study conducted in this research do not accommodate the data from the subfamily Scomberoidinase as the unavailability of mitogenomic sequences. Future studies involving the mitogenomic data of Scomberoidinae are required for a better understanding of the phylogenetic and evolutionary relationship between major groups within the Carangidae. Mitogenomic phylogeny is also influenced by the habitat characteristics of each group, as habitat-specific selection and adaptation in mitochondrial coding genes has recently been reported. The subfamilies Trachinotinae, Naucratinae, and Caranginae consist of species with differential habitat preferences (pelagic, benthopelagic, and reef-associated) and schooling behavior. The mutational patterns in the protein-coding regions of these fish need to be further analyzed for adaptive variation and selection and correlated with habitat characteristics to understand the forces of selection, if any.

## CHAPTER 4

# Genetic stock structure analysis and historical demography of the Indian scad, *D. russelli* from the Indian Ocean using mitochondrial and nuclear markers.

## 4.1 Introduction

To identify the genetic changes occurring within in a population is of paramount importance as it may have drastic and long-term effect on a population. For a fishery resource which are subjected to variation in oceanic parameters, differential harvesting and migration, determining the genetic changes at the population level of such a species is vital, as it may holds the key to developing conservation planning. Thus, understanding the population subdivision of fish species is crucial, since the conservation methods focus mainly on whether the population being considered is a single or distinct evolutionary unit. Furthermore, determining the underlying mechanism that result in intra and interspecific variation as well as historical and contemporary demographic process which establish the spatial pattern of genetic diversity together holds the key to establishing conservation planning.

Pelagic species are usually assumed to lack genetic spatial structure due to their widespread distribution, large population size, high fecundity, production of numerous pelagic larvae, and ease of migration (Muths et al., 2013). Several studies from Indian ocean spanning the wide range of pelagic species reported to have lack of genetic structuring (Chiang et al., 2008; Kumar et al., 2012; Muths et al., 2013). Many other studies identified species with apparent level of genetic structuring ( Lu et al., 2006; Habib et al., 2012; Divya et al., 2015; Sebastian et al., 2017). It has been reported that several species of migratory species have undergone staggering declines in their population worldwide (Haro et al., 2000; Hutchings 2005; Schoen 2021). Failure to arrest these declines will ultimately lead to the loss of these species from their current habitats and will have evolutionary consequences. Thus, understanding the population structure of any species and its movement pattern are of paramount importance for effective conservation and judicial management of fishery resources.

The species, *D. russelli* has gained attention on stock assessment especially on reproductive and biological characteristics from various coast of India (Murty 1991; Reuben et al., 1992; Raje 1997; Balasubrahmanian and Natarajan 2000; Manojkumar 2005; Poojary et al., 2015; Kalhoro et al., 2017). The food and feeding habit of *D. russelli* has been studied from north-west coast of India (Poojary et al., 2010). A study regarding its length weight relationship has been carried out from the north-west coast of India (Jaiswar et al., 1993). Apart these studies, the genetic information on this species is still scarce from Indian waters. The species has extensively studied from IMA for its population structure based on sequence polymorphism

of mitochondrial and nuclear DNA (Perrin and Borsa 2001; Rohfritsch and Borsa 2005). Sen et al. (2011) assessed the stock structure of this species from Indian waters based on truss morphometric analysis and found out separate stocks of this species from the east and west coast of India. But no attempts have been carried out to unravel the genetic basis for this observed phenotypic discreteness. This need to be established for effective management recommendations for the contemporary species in different geographical areas across Indian Ocean. Hence, we investigated the genetic population structure of Indian scad mackerel collected from five locations using mitochondrial and nuclear markers. We also carried out a comparative study by extending the geographic area to IMA in order to identify is there any genetic break over the geographical area.

## 4.2 Review of Literature

Earlier, the catch and effort data based on spatial and temporal distribution of species obtained from commercial fisheries can be used as a strong indicator for understanding the structure and behaviour of a fish population to some extent. For example, the geographic differences in relative abundance or distribution (Marsh-Matthews and Matthews 2000; Munday 2002), spawning time (Otterå et al., 2012; Reglero et al., 2012), age (Hodge and Bellwood 2015) and composition (Floeter et al., 2001) etc. are reported for several fish species.

Early research on the reproductive history of *D. russelli* indicated that different spawning period which occasionally overlapped in various coast of India (Sreenivasan 1981; Murty 1991; Reuben et al., 1992; Raje 1997; Balasubrahmanian and Natarajan 2000; Manojkumar 2007; Poojary et al., 2015). The species seems to show a particular shift in food choice as they grow, according to several studies based on food and feeding habits on this species from different coasts of India (Sreenivasan 1974; Raje 1997; Manojkumar 2007; Poojary et al., 2010). Studies on stock assessment of this species from south west coast of India (Balasubrahmanian and Natarajan 2000; Manojkumar 2007) reported two recruitment pulses for the species: one was a major season compared to the latter. However, reports of various types of recruitment patterns for the species around the world have also been reported (Ingles and Pauly 1984; Dalzell 1988; Atmaja 1998). Similarly, the analysis of the stock assessment of the species from Pakistani waters (Kalhoro et al., 2017) indicated that the stock of *D. russelli* is in an overexploited state in the northern parts of the Arabian Sea coast of Pakistan. While the usage status of Indian Scad does not exceed the maximum limit based on data from Tasikagung fishing port of Rembang

(Khasanah et al., 2020). A study based on morphological character analysis from Indonesia (Lelono et al., 2021) suggested a three-stock structure of *D. russelli*. The population genetic structure of this species has been extensively studied from the Indo Malay Archipelago: First, Perrin and Borsa (2001) identified two distinct clades of *D. russelli* with heterogenous geographical distribution based on analysis of mitochondrial C*yt b* sequences. Later, Rohfrtisch and Borsa (20005) identified three geographically distinct lineages based on the combined data of mitochondrial (*Cyt b*) and nuclear (*Aldolase b*) markers. Further, the stock structure of this species from the Indian Ocean has been assessed based on truss morphometric analysis by using 23 variables and identified separate stocks from east and west coast of India (Sen et al., 2011). So far, no studies have been conducted to assess the population structure of these species at the molecular level from the Indian Ocean.

## 4.3 Materials and Methods

### 4.3.1 Study area and sample collection

A total of 125 *D. russelii* individuals were collected between 2019 and 2020 from five locations representing the east and west coasts of India. The east coast sampling sites included Odisha (Puri) and Tamil Nadu (Chennai), while the west coast sites comprised Gujarat (Veraval), Karnataka (Mangalore), and Kerala (Cochin). Sufficient nautical distance was maintained between the sampling locations to capture of genetic variability. For each individual, a piece of caudal fin base tissue was collected and preserved in 95% ethanol until DNA isolation.

### 4.3.2 DNA isolation and PCR amplification

Genomic DNA was isolated from tissue using the standard phenol- chloroform protocol (Sambrook and Russell 2001). In order to facilitate comparative analysis, a novel primer set specific for a 910 bp segment of the mitochondrial *Cyt b* gene spanning the regions analyzed by Rohfritsch and Borsa (2005) was designed. The primer sequences were as follows: 5'-AATCTCCGTAAAACCCACCC-3'(forward) and 5'-AAAAACTAGGAATAGGAGGAAGT-3' (reverse). The primers were designed based on the aligned complete *Cyt b* gene sequences of carangids using Geneious R7 (Biomatters Ltd., New Zealand). The PCR reaction mixture consisted of 15.6 µl sterile deionized water, 2µl 10X buffer, 10 mM of each dNTPs, 10 µM of each oligonucleotide, 1 unit of Taq DNA polymerase and ~50 ng of genomic DNA as template Amplification conditions were: initial denaturation at 94 °C (4 min); 32 cycles of 94 °C (30 sec), 55.4 °C (30 sec), 72 °C (1 min 10 sec) and a final extension at 72 °C (10 min) before the

reaction termination at 4 °C. Amplification of this 20 µl reaction volume was performed in a Biorad T100 thermal cycler (Bio-Rad, USA).

Intron 1 of the aldolase b (*DrAldoB1*) gene was targeted with the following primers; 5-GCTCCAGGAAAGGGAATCCTGC-3 (forward) and 5-CTCGTGGAAGAAGATGATCCCGCC-3 (reverse) (Hassan et al., 2002). PCR amplification was performed in a Biorad T100 thermal cycler (Bio-Rad, USA) with a final volume of 25µl, consisting of 12.5 µl of 2X EmeraldAmp® GT PCR Master Mix (TaKaRa, Shiga, Japan), 0.3 µl of each primer (10mM each), 2.0 µl of genomic DNA (50 ng/mL) followed by nuclease-free water to achieve final reaction volume. Thermal cycling conditions were as follows: initial denaturation at 94 °C for 4 min, followed by 35 cycles of 30 sec denaturation at 94 °C, 12 sec annealing at 52 °C, 20 sec extension at 72 °C followed by final extension of 3 min at 72 °C. DNA amplification products were then separated in 1.5% agarose gels at 90 V in 1X Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide and visualized using a gel documentation system (Bio-Rad, USA). All PCR products were then sequenced using sanger sequencing in both the forward and reverse directions. The nucleotide sequences were checked visually to ensure that the sequence information was consistent in both directions. The sequences were also verified by comparison with known sequences from GenBank.

### 4.3.3 Data analysis

Both forward and reverse raw sequences were edited and assembled after removing the ambiguous bases in MEGA X (Kumar et al., 2018). A total of 933 bp sequences of *Cyt b* and 212 bp sequences of *DrAldoB1* were obtained for the subsequent analysis. Further, *Cyt b* (accession numbers: AF307494-AF307510, AF515757-AF515759) and *DrAldoB1*(accession numbers: AF500103-AF500105) gene sequences deposited from IMA were also retrieved for comparative analysis. Prior to the data analysis, the programme PHASE (Stephens et al., 2001) implemented in DnaSP6 (Rozas et al., 2017) was used to resolve heterogenous sites in *DrAldoB1* sequences to reconstruct haplotypes.

### 4.3.4 Genetic diversity analysis

Genetic distance within and among populations was estimated using K2P model in MEGA X (Kumar et al., 2018). The complete aligned dataset was inspected for nucleotide variable sites, parsimony informative sites, haplotype number, haplotype frequencies and nucleotide

frequencies in DnaSP6 (Rozas et al., 2017). The ratio of transition to transversion were analysed using MEGAX (Kumar et al., 2018).

### 4.3.5 Population genetic structure analysis

Population pairwise $F_{ST}$ values were calculated using ARLEQUIN v3.5 (Excoffier and Lischer 2010). Hierarchical analysis of molecular variance (AMOVA) was executed to estimate population subdivision and structuring at different hierarchical level using ARLEQUIN v3.5 (Excoffier and Lischer 2010). For the Indian Ocean population, a hierarchical AMOVA analysis was performed by partitioning the variance among and within populations. i) one gene pool comparison considering the whole population as a single gene pool, ii) two gene pools [West coast (Cochin, Mangalore and Veraval); East coast (Chennai and Puri)] and iii) three gene pools [North West (Veraval); South West (Mangalore and Cochin); East (Chennai and Puri) iv) four gene pools [North West (Veraval); South West (Mangalore and Cochin); South East (Chennai) and North East (Puri)].

For the comparative analysis, the entire data set is divided into two groups: one comprises the populations from Indian ocean and next encompass the entire population from the IMA. The variance among populations and the relative contribution of variance were estimated at three different levels; $F_{ST}$ (variation between populations within total), $F_{SC}$ (variation among populations within region) and $F_{CT}$ (variation among regions within total) (Excoffier and Lischer 2010). In addition, the spatial structure was examined using spatial analysis of molecular variance (SAMOVA) v2.0 (Schneider and Excoffier 1999; Dupanloup et al., 2002). Geneflow (Nm) between populations based on both haplotype and sequence statistics was estimated according to Nei (1973) and Hudson et al. (1992) using DnaSP6 (Rozas et al., 2017). Genetic differentiation estimates based on $H_{ST}$, $N_{ST}$ and $K_{ST}^{*}$ were determined in DnaSP6 (Rozas et al., 2017). The isolation by distance or mantel test was conducted to confirm the relationship between genetic distance and geographical distance (Mantel 1967).

### 4.3.6 Phylogenetic analysis

Phylogenetic relationship among haplotypes were examined by constructing a phylogenetic tree based on Bayesian inference (BI) in MrBayes (Ronquist et al., 2012) with 1,000,000Markov Chain Monte Carlo (MCMC) generations. Posterior distributions were sampled for every 10000 generations. GenBank sequence of *D. macarellus* (Accession No. NC026718) was employed as outgroup in the analysis of phylogenetic relationship using *Cyt*

*b* and sequences of *D. macarellus* (Accession No. AF500103) and *D. macrosoma* (Accession No. AF500104, AF500105) were used as outgroup to construct phylogenetic tree using *Aldolase b*. A phylogenetic network of all haplotypes was constructed to explore haplotype relationships based on median joining calculations in a minimum spanning network (MSN) implemented in the population analysis with reticulate trees (PopARTv1.7) (Bandelt et al., 1999; Leigh and Bryant 2015).

### 4.3.7 Demographic history

The historical demographic expansion of each population was tested using Tajima's D (Tajima 1989) and Fu's F (Fu 1997) statistics in ARELIQUIN v3.5 (Excoffier and Lischer 2010). In addition, Harpending's raggedness index, *Hri* (Harpending 1994) implemented in ARLEQUIN v3.5 (Excoffier and Lischer 2010) and mismatch distribution (Slatkin and Hudson 1991; Rogers and Harpending 1992) implemented in DnaSp6 (Rozas et al., 2017) were applied to find out whether the sampled populations were demographically stable or expanding or decreasing over time.

The precise time for expansion was calculated using the equation $\tau = 2ut$ (Rogers and Harpending 1992); where t is the expansion time. 2u was calculated using the equation $2u= \mu k$ (Nei and Tajima 1981) where $\mu$ is the mutation rate which is 1-2%/ site/year for the mitochondrial *Cyt b* gene in fishes (Carangidae) (Rousset 1997; Johns and Avise 1998; Cárdenas et al., 2005) and k is the number of nucleotide covered in the data (307 bp).

## 4.4 Results

### 4.4.1 Genetic Diversity Analysis

#### 4.4.1.1 Mitochondrial DNA (*Cyt b*) analysis

A total of 125 individuals sampled from five locations along the Indian coast successfully amplified for the *Cyt b* gene. After aligning and editing ambiguous sequences, a final sequence length of 933 bp was obtained. The aligned dataset revealed a total of 58/933 (6.21%) variable sites, with 14/993 sites (1.50%) being parsimoniously-informatively defined 38 haplotypes. All haplotypes were deposited in GenBank under accession numbers OR188721 to OR188758. The descriptive statistics of each population from Indian ocean are given in Table 4.1. The IMA sequences previously reported were also included in the study (Rohfritsch and Borsa 2005) bringing the total number of sequence to 583. We trimmed our sequences to match with those retrieved from GenBank resulting in a final length of 307 bp. These data were used for further

analysis. Altogether the dataset revealed 30 variable sites (30/307), of which 14 parsimony informative and 16 singleton variable sites defined 27 haplotypes (Table 4.2)

Table 4.1 Parameters of variability estimates for *D. russelli* populations from Indian Ocean based on mitochondrial *Cyt b.*

| | Indian Ocean | | | | |
|---|---|---|---|---|---|
| | East coast | | West Coast | | |
| | Chennai | Puri | Cochin | Mangalore | Veraval |
| Number of samples | 25 | 25 | 25 | 25 | 25 |
| Number of haplotypes | 12 | 8 | 5 | 7 | 17 |
| Number of polymorphic sites | 10 | 10 | 4 | 26 | 25 |
| Haplotype diversity, Hd | 0.80667 | 0.66333 | 0.4233 | 0.43000 | 0.90333 |
| Nucleotide diversity | 0.00117 | 0.00124 | 0.00071 | 0.00231 | 0.00283 |
| Tajimas D | -1.15753 | -1.51406 | 0.00000 | -2.30550 | -1.51406 |
| Fu's Fs | -1.06131 | -2.12796 | 0.00000 | 0.91456 | -2.12796 |
| *Hri* index | 0.71200 | 0.49861 | 0.00000 | 0.60492 | 0.49861 |

The genetic diversity or distance was converted to percentage and revealing that majority of the intrapopulation genetic diversity is 0% with the exception of Toli-Toli and Makassar which have 1% genetic distance. Makassar and Toli-Toli from the IMA were the most divergent of all, with at least 1% of genetic distance with the remaining populations and 1% difference from each other. The genetic distance of 2% was mainly observed between Indian Ocean and IMA populations (Mangalore and Toli-Toli: Mangalore and Makassar; Puri and Tili-Toli). Furthermore, all other Indian Ocean populations are genetically separated from the IMA by 1%. The majority of $F_{ST}$ values observed in the IMA were significantly different ($P<0.05$). In addition, significant differences in the $F_{ST}$ values ($P<0.05$) were also observed between the

populations from the IMA and Indian Ocean. Nevertheless, non-significant values (P>0.05) were detected between populations from the Indian ocean.

Table 4.2 Distribution of haplotypes for 15 populations of *D. russelli* based on *Cyt b* gene sequence data.

| Region | East coast of India | | West coast of India | | | Indo Malay Archipelago | | | | | | | | | | Total | Haplotype frequency (%) Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population | Chennai | Puri | Kochi | Mangalore | Veraval | Kelang | Carita | Labuan | Tambelan | Pekalongan | Kinabalu | Sandakan | Toli-Toli | Makassar | Arafura | | |
| Haplotype 1 | | | | | | 1 | | | | | | | | | | 1 | 0.17 |
| Haplotype 2 | | | | | | | | | | | | 1 | | | | 1 | 0.17 |
| Haplotype 3 | | | | | | | | | | | | 1 | | | | 1 | 0.17 |
| Haplotype 4 | | | | | | | | | | | | | 22 | 14 | | 36 | 6.20 |
| Haplotype 5 | | | | | | | | | | 1 | | | | | | 1 | 0.17 |
| Haplotype 6 | | | | | | | 1 | 2 | | | | | | | | 3 | 0.51 |
| Haplotype 7 | | | | | | | | 1 | | | | | | | | 1 | 0.17 |
| Haplotype 8 | | | | | | | | | 1 | | 1 | | | | | 2 | 0.34 |
| Haplotype 9 | | | | | | | 1 | 1 | 1 | | 1 | | | | | 4 | 0.70 |
| Haplotype 10 | | | | | | | | | 1 | | | | | | | 1 | 0.17 |
| Haplotype 11 | | | | | | | | | | 1 | | | | | | 1 | 0.17 |
| Haplotype 12 | | | | | | | | | | 1 | | | | | | 1 | 0.17 |
| Haplotype 13 | | | | | | | | | | | | 1 | | | | 1 | 0.17 |
| Haplotype 14 | | | | | | | 1 | | | | | | | | | 1 | 0.17 |
| Haplotype 15 | | | | | | | | | | | | 1 | | | | 1 | 0.17 |
| Haplotype 16 | | | | | | | | 1 | | | | | | | | 1 | 0.17 |
| Haplotype 17 | | | | | | | | | 1 | | | | | | | 1 | 0.17 |
| Haplotype 18 | | | | | | 1 | 1 | | | | | 1 | | | | 3 | 0.51 |
| Haplotype 19 | | | | | | 10 | 38 | 72 | 48 | 64 | 35 | 61 | 8 | 8 | 51 | 395 | 67.75 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplotype 20 | | | | | | | | 2 | | | | | | | | | 2 | 0.34 |
| Haplotype 21 | 24 | 23 | 25 | 23 | 23 | | | | | | | | | | | | 118 | 20.24 |
| Haplotype 22 | | | | 1 | | | | | | | | | | | | | 1 | 0.17 |
| Haplotype 23 | | | | 1 | | | | | | | | | | | | | 1 | 0.17 |
| Haplotype 24 | | | | | 1 | | | | | | | | | | | | 1 | 0.17 |
| Haplotype 25 | 1 | | | | 1 | | | | | | | | | | | | 2 | 0.34 |
| Haplotype 26 | | 1 | | | | | | | | | | | | | | | 1 | 0.17 |
| Haplotype 27 | | 1 | | | | | | | | | | | | | | | 1 | 0.17 |
| Total | 25 | 25 | 25 | 25 | 25 | 12 | 42 | 79 | 52 | 67 | 37 | 66 | 30 | 22 | 51 | 583 | | |

#### 4.4.1.2 Nuclear DNA (*DrAldoB1*) analysis

A total of 125 individuals (250 alleles) of *D. russelli* from Indian Ocean were assayed for 212 bp of intron 1 of *Aldolase b* (*DrAldoB1*) gene. 36 unique alleles were identified of which 33 were exclusively from the Indian Ocean (Table 4.3) and the remaining three were from IMA. The sequences were deposited in GenBank under accession numbers OR546246 to OR546278. Overall, the haplotype diversity was 0.3786 and nucleotide diversity was 0.01. The observed intrapopulation genetic diversity was varied from 0.1% (Veraval) to 1% (Puri and Mangalore). The inter population genetic diversity was negligible with a range between 0.3% to 1%. The global $F_{ST}$ value observed was low (0.052). Pairwise $F_{ST}$ analysis revealed a lack of genetic structure among the areas studied from the Indian Ocean and was not significant. However significant values were obtained between IMA and Indian Ocean populations.

### 4.4.2 Population structure

#### 4.4.2.1 Within Indian Ocean analysis

AMOVA analysis undertaken with mitochondrial and nuclear markers shown lack of significant genetic structure in *D. russelli* (Table 4.4). Additional genetic differentiation analysis revealed that the estimates based on $H_{ST}$ (0.006), $N_{ST}$ (0.002) and $K_{ST}^{*}$ (0.001) were all very low and none of them were significant. This also suggest a high level of gene flow among sampled *D. russelli* populations from Indian Ocean (Nm= 207.34 for haplotype-based statistics and Nm= 83.97 for sequenced based statistics). The mantel test showed no significant correlation between genetic distance (pairwise $F_{ST}$ values) and geographical distance ($r=0.063$, $p>0.05$) among the tested population. Overall, four and three populations out of five were significant with negative values of Tajimas' D and Fu's Fs (Table 4.1) suggesting historical population expansion at these sites associated with an excess of recent mutations or rare alleles (Fu 1997). The Harpending raggedness index (*Hri* =0.46, SSD= 0.03, P=0.02) corroborated these results. Mismatch analysis detected unimodal distribution providing strong evidence for sudden population expansion (Figure 4.1). The minimum spanning network (MSN) produced a reticulation of 38 haplotypes (Figure 4.2). Phylogenetic analysis based on mitochondrial and nuclear markers revealed no obvious phylogeographic pattern separating the 5 samples of *D. russelli*.

Table 4.3 Unique alleles of *Aldolase b* detected in *D. russelli* from Indian ocean.

| Alleles | Population | | | | | Total |
|---|---|---|---|---|---|---|
| | Chennai | Puri | Cochin | Mangalore | Veraval | |
| 1 | | | | | 2 | 2 |
| 2 | | | | | 2 | 2 |
| 3 | 30 | 46 | 39 | 36 | 46 | 197 |
| 4 | | 1 | | | | 1 |
| 5 | | 1 | | | | 1 |
| 6 | | 1 | | | | 1 |
| 7 | | 1 | | | | 1 |
| 8 | | | | 2 | | 2 |
| 9 | | | | 2 | | 2 |
| 10 | | | | 1 | | 1 |
| 11 | | | | 1 | | 1 |
| 12 | | | | 2 | | 2 |
| 13 | | | | 2 | | 2 |
| 14 | | | | 2 | | 2 |
| 15 | | | | 2 | | 2 |
| 16 | 6 | | | | | 6 |
| 17 | 1 | | | | | 1 |
| 18 | 1 | | | | | 1 |
| 19 | 3 | | | | | 3 |
| 20 | 1 | | | | | 1 |
| 21 | 2 | | | | | 2 |
| 22 | 2 | | | | | 2 |
| 23 | 1 | | | | | 1 |
| 24 | 1 | | | | | 1 |
| 25 | 1 | | | | | 1 |
| 26 | 1 | | | | | 1 |
| 27 | | | 1 | | | 1 |
| 28 | | | 1 | | | 1 |
| 29 | | | 2 | | | 2 |
| 30 | | | 2 | | | 2 |
| 31 | | | 3 | | | 3 |
| 32 | | | 1 | | | 1 |
| 33 | | | 1 | | | 1 |
| Total | 50 | 50 | 50 | 50 | 50 | 250 |

Table 4.4 Results of the analysis of molecular variance (AMOVA) for *D. russelli* showing F-statistics analysis of mitochondrial *Cyt b* and *DrAldoB1*

| *Cyt b* | | | |
|---|---|---|---|
| Hierarchical level | Variation (%) | F-statistic | P value |
| **Among IO** | | | |
| Among all regions ($F_{CT}$) | 1.09 | 0.01090 | ns |
| Among populations within region ($F_{SC}$) | -0.06 | -0.00061 | ns |
| Among individuals within populations ($F_{ST}$) | 98.97 | 0.01030 | ns |
| **Between IO and IMA** | | | |
| Among all regions ($F_{CT}$) | 72.09 | 0.72094 | * |
| Among populations within region ($F_{SC}$) | 14.14 | 0.50668 | * |
| Among individuals within populations ($F_{ST}$) | 13.77 | 0.86233 | * |
| *DrAldoB1* | | | |
| **Among IO** | | | |
| Among all regions ($F_{CT}$) | -0.11 | -0.00114 | ns |
| Among populations within region ($F_{SC}$) | 5.37 | 0.05364 | ns |
| Among individuals within populations ($F_{ST}$) | 94.74 | 0.05256 | ns |
| **Between IO and IMA** | | | |
| Among all regions ($F_{CT}$) | 80.02 | 0.80203 | * |
| Among populations within region ($F_{SC}$) | 14.80 | 0.14802 | * |
| Among individuals within populations ($F_{ST}$) | 5.98 | 0.52561 | * |

IMA: Indo-Malay Archipelago; IO: Indian Ocean.

ns, not significant (P>0.05).

*P<0.01

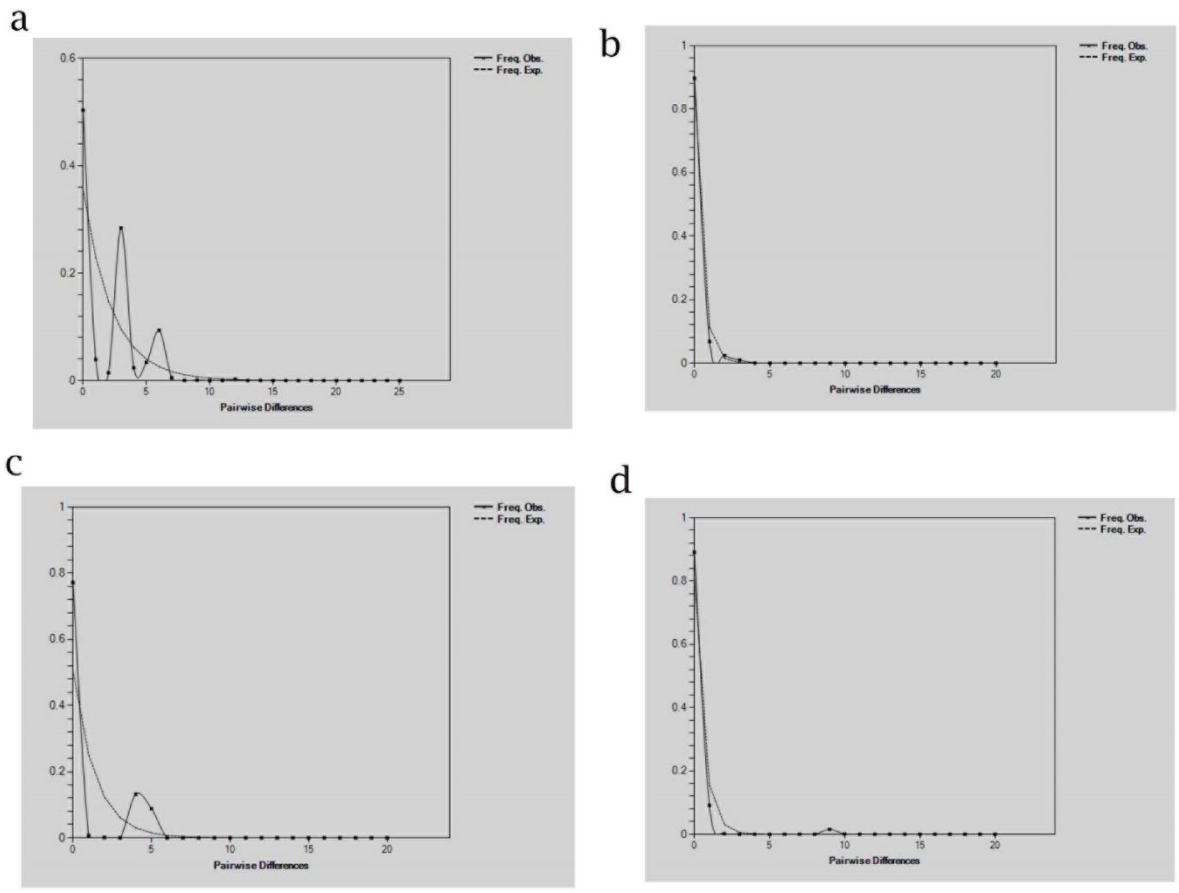


Figure 4.1 Mismatch distribution (pairwise difference) for *D. russelli* for (a) whole population (b) IMA lineage 1 (c) IMA lineage 2 (d) IO lineage.

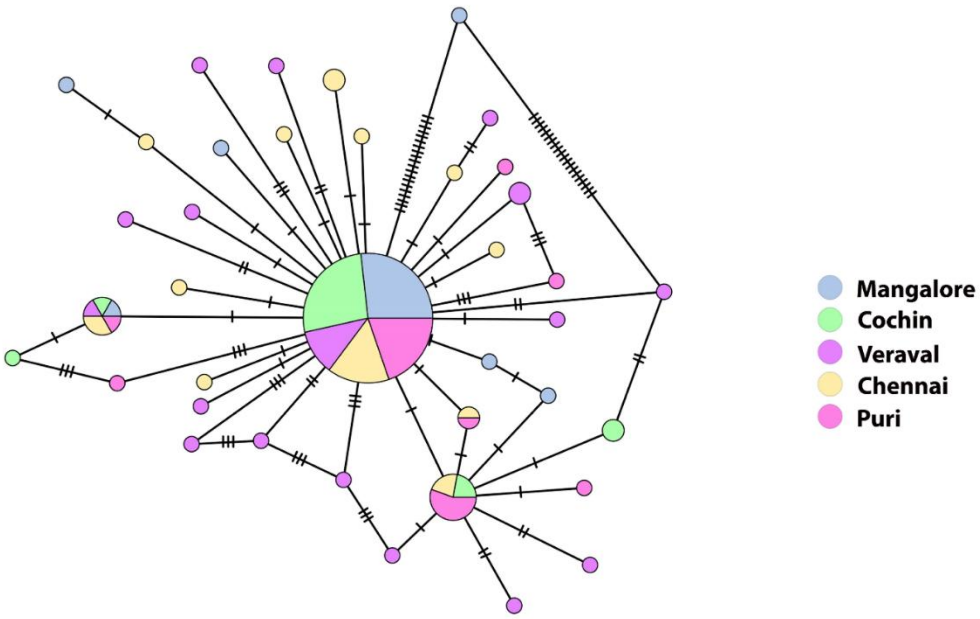


Figure 4.2 Minimum spanning network (MSN) inferred from mtDNA *Cyt b*. Coloured close circles represents different regions (refer to legend)

#### 4.4.2.2 Between Indian Ocean and IMA analysis

The AMOVA analysis was also performed on the 15 populations (10 from the IMA and 5 from the Indian Ocean) that were subdivided into two geographical groups (Group 1: Indian Ocean; Group 2: IMA) to determine whether there is any genetic structuring over the geographical area. AMOVA analysis based on *Cyt b* and *DrAldoB1* revealed that genetic differentiation was significant at all levels (Table 4.4), with 72.09% of total variation attributed to variation among regions/groups, implying significant differentiation across geographical areas. In addition, genetic differentiation estimates were analysed by dividing the whole population into two: Indian ocean and IMA. All values generated ($H_{ST}$=0.60, $N_{ST}$ = 0.79 and $K_{ST}^{*}$ = 0.54) were high and significant. The result from the SAMOVA were consistent with other analysis, with k=2 showing the highest $F_{CT}$ value ($F_{CT}$ = 0.8362, $p<0.05$) clearly demonstrating the existence of two genetically distinct *D. russelli* stocks among the populations under study, namely the IMA and Indian ocean populations. The mantel test showed significant correlation between genetic differentiation (pairwise $F_{ST}$) and geographical distance (r =0.11, $p<0.05$) among the tested population.

### 4.4.3 Phylogenetic relationship

The Bayesian phylogenetic tree revealed 3 mitochondrial lineages (Figure 4.3). The two lineages were haplogroup M and A formerly identified by Perrin and Borsa (2001). The third lineage consists populations from the Indian ocean. In addition, Perrin and Borsa's M haplogroup formed a sister lineage with the populations from Indian ocean. The Bayesian phylogenetic tree produced from *DrAldoB1* formed 2 lineages representing an Indian Ocean lineage and an IMA lineage.

### 4.4.4 Haplotype network diagram

The haplotype network diagram (Figure 4.4) derived from mitochondrial cytochrome b gene partial sequences delineated 3 major clusters similar to the phylogenetic tree: haplogroup A, haplogroup M and Indian Ocean populations. The rarer haplotypes from IMA, h1, h2 and i that comprises the M haplogroup forms a distinct cluster separated from haplotype M- the most frequent haplotype in the haplogroup M- by 4 genetic mutations, supporting the finding of Perrin and Borsa (2001). The haplogroup M separated from haplogroup A by 6 mutational steps. The cluster formed by Indian Ocean population was separated from haplogroup A and M by 8 and 4 mutational steps respectively.

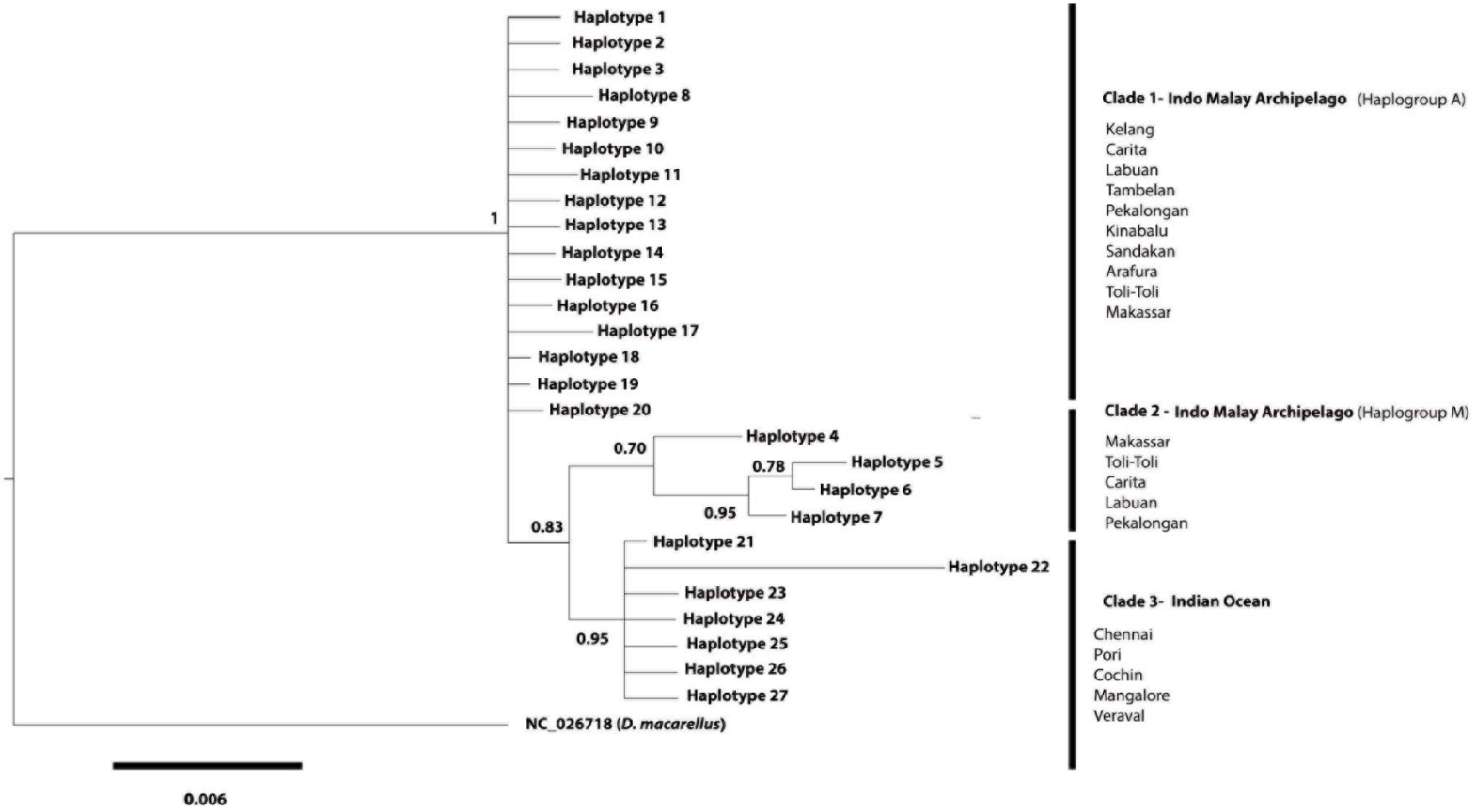


Figure 4.3 Bayesian Inference phylogenetic relationships among 27 mtDNA *Cyt b* haplotypes in *D. russelli.* Bayesian posterior probabilities are superimposed with each node. The phylogenetic tree was generated using MrBayes 3.2.7

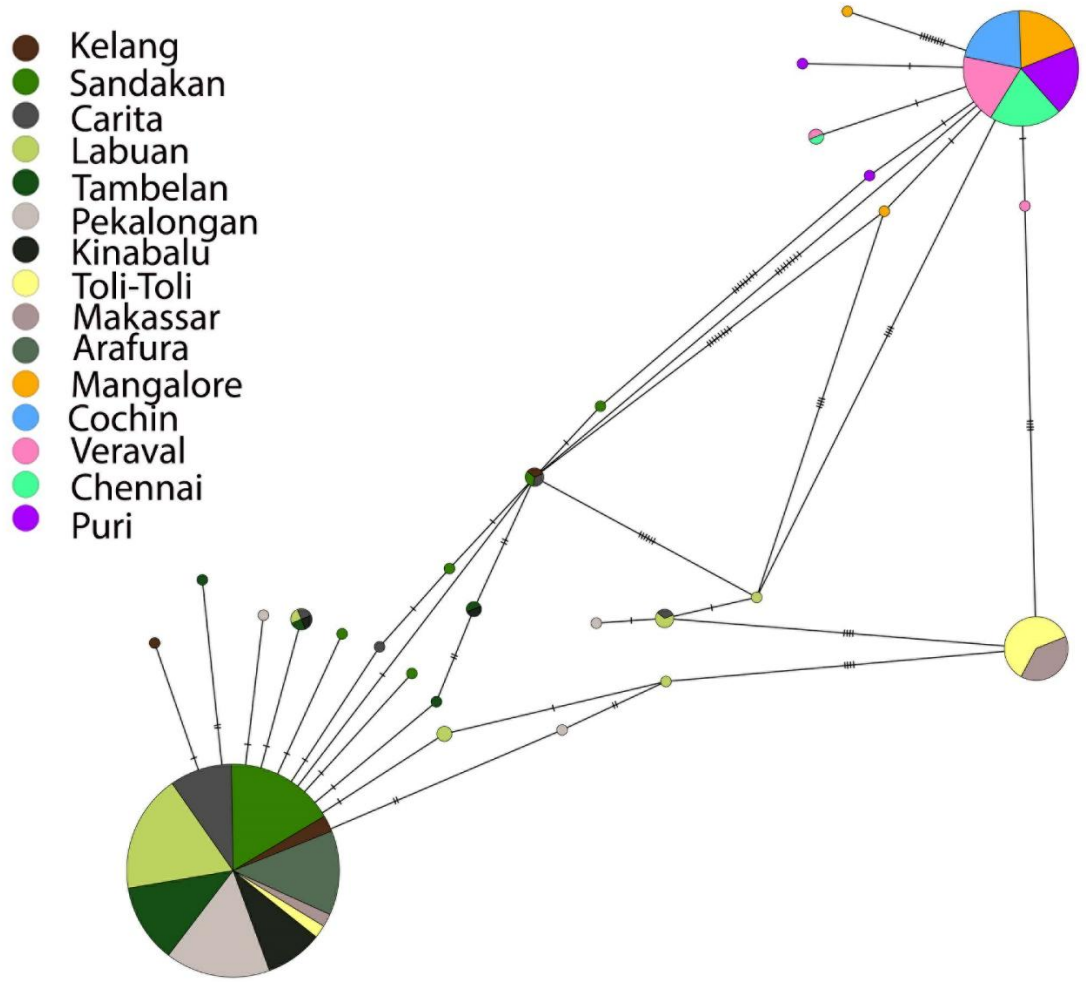


Figure 4.4 Minimum spanning network inferred from mitochondrial *Cyt b.* Colored circles represent different regions (refer to legend).

### 4.4.5 Demographic history

Overall, the 11 and 10 populations out of the total populations tested were significant with negative Tajimas D and Fu's Fs (Table 4.5) respectively indicating a recent historical directional selection (selective sweep) or population expansion (Tajima 1989) at these sites. In addition, the Harpending raggedness index, *Hri* = 0.5, SSD= 0.04 (P=0.2) corroborated this finding with the recent population growth hypothesis (Harpending 1994). Furthermore, based on the mismatch distribution of the combined population, past population demography of *D. russelli* revealed three highly divergent peaks, representing the three lineages (Figure 4.1 a). The IMA lineage 1 and the Indian Ocean (IO) lineage, on the other hand, has a unimodal distribution, indicating a rapid expansion of its population.

Table 4.5 Sample size, population genetic statistics and historical demographic analysis of 15 *D. russelli* populations based on mitochondrial *Cyt b* gene sequences.

| | Indian Ocean | | | | | Non-Indian Ocean | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | East Coast | | West Coast | | | IMA | | | | | | | | | |
| | Chennai | Puri | Cochin | Mangalore | Veraval | Kelang | Carita | Labuan | Tambelan | Pekalongan | Kinabalu | Sandakan | Toli-Toli | Makassar | Arafura |
| N | 25 | 25 | 25 | 25 | 25 | 12 | 42 | 79 | 52 | 67 | 37 | 66 | 30 | 22 | 51 |
| Number of haplotypes | 2 | 3 | 1 | 3 | 3 | 3 | 5 | 6 | 5 | 4 | 3 | 6 | 2 | 2 | 1 |
| Number of polymorphic sites | 1 | 2 | 0 | 10 | 2 | 2 | 8 | 9 | 6 | 10 | 4 | 5 | 6 | 6 | 0 |
| Haplotype diversity, Hd | 0.0800 | 0.15667 | 0.00 | 0.15667 | 0.15667 | 0.31818 | 0.18351 | 0.16975 | 0.14932 | 0.08820 | 0.10661 | 0.14658 | 0.440460 | 0.48485 | 0.000 |
| Nucleotide diversity | 0.00026 | 0.00052 | 0.00 | 0.00261 | 0.00052 | 0.00109 | 0.00140 | 0.00177 | 0.00087 | 0.00098 | 0.00070 | 0.00068 | 0.00791 | 0.000948 | 0.000 |
| Tajimas D | -1.15753 | -1.51406 | 0.00000 | -2.30550 | -1.51406 | -1.45138 | -2.17642 | -1.81405 | -2.01660 | -2.33128 | -1.88391 | -1.83587 | 1.72038 | 2.37829 | 0.00000 |
| Fu's Fs | -1.06131 | -2.12796 | 0.00000 | 0.91456 | -2.12796 | -1.32484 | -2.61585 | -2.49722 | -3.82287 | -1.84208 | -1.37648 | -6.31047 | 7.19054 | 7.43462 | 0.00000 |
| *Hri* index | 0.71200 | 0.49861 | 0.00000 | 0.60492 | 0.49861 | 0.22658 | 0.48821 | 0.58186 | 0.60829 | 0.78190 | 0.71515 | 0.59213 | 0.68190 | 0.73554 | 0.00000 |

Bayesian skyline plot analysis revealed a recent expansion in overall effective population size (Figure 4.5 a). This can be explained by growth in different lineages. The growth in IMA+IO lineage and IO lineage occurred approximately around 300 thousand years ago (KYA) (Figure 4.5 c, d). The IMA lineage 2 showed a population expansion around 500 KYA. The IMA lineage 1 showed a gradual population increase over the last 700 KYA (Figure 4.5 b). The estimated time of demographic and spatial expansion for the major lineages, 1 (IMA lineage 1) and 2 (IMA lineage 2 + IO lineage) using the formula $\tau = 2\ \mu t$ are estimated to occur 8,58,934 - 4,46,254 years ago and 4,57,680 – 4,46,254 years ago respectively. Therefore, the spatial expansion coincided with demographic expansion. Furthermore, the spatial and demographic expansion of IO lineage occur between 6,11,285 – 3,17,589 years ago. In addition, the demographic expansion of lineage 2 (IMA lineage 2) occur between 7,93,159 -1,52,664 years ago whereas the spatial expansion of its population was between 3,66,771 – 1,90,553 years ago. Therefore, this lineage experienced demographic expansion following spatial expansion. These results are almost in consistent with the results from the Bayesian Skyline plot suggesting a demographic expansion in the *D. russelli* population during the Pleistocene era.

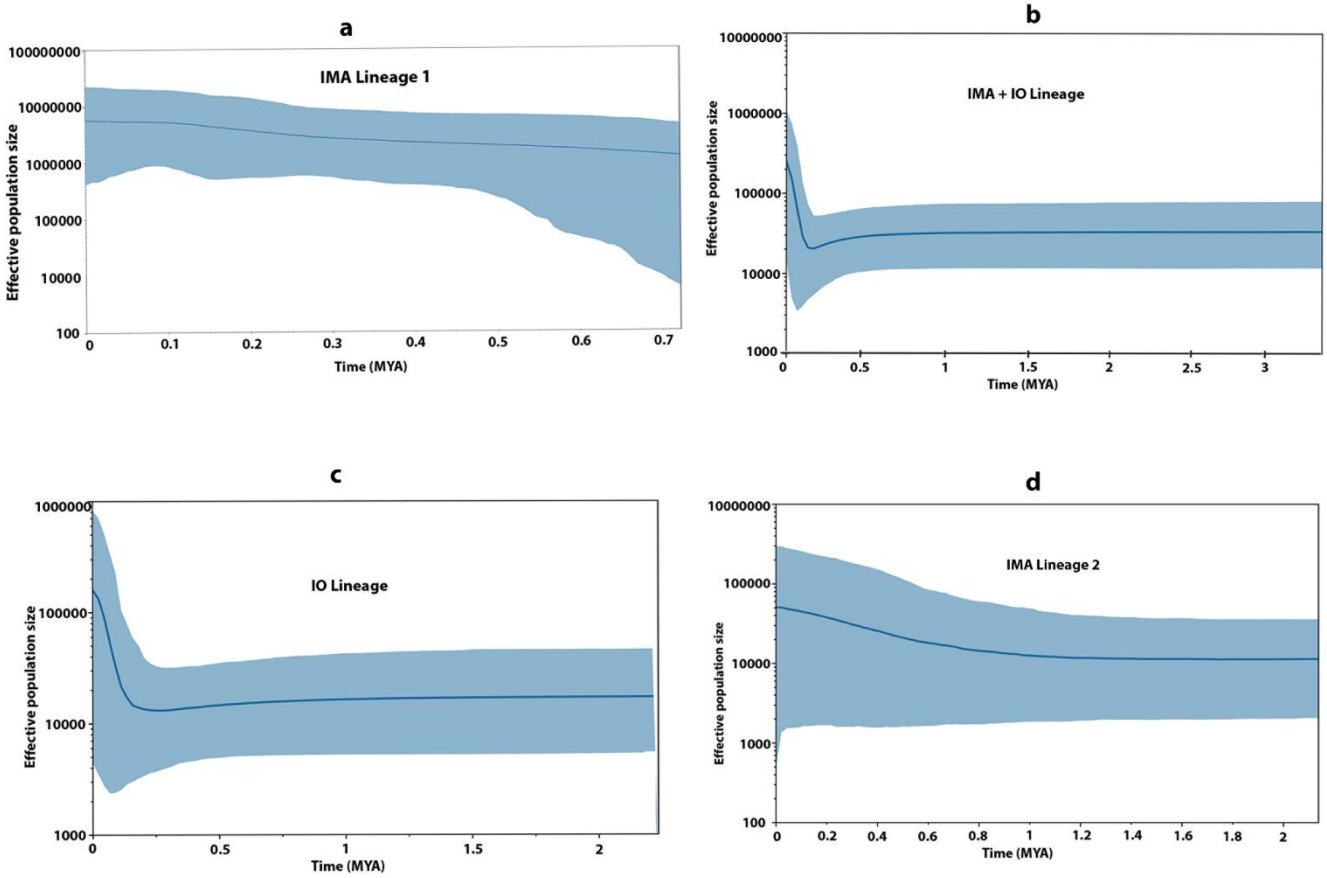


Figure 4.5 (a) The Bayesian Skyline plot (BSP) of the effective population size for mtDNA *Cyt b* for *D. russelli*. (b) The BSP for IMA lineage 1. (c) The BSP for IMA+IO lineage. (d) The BSP for IO lineage. (e) The BSP for IMA lineage 2.

## 4.5 Discussion

Genetic data based on mitochondrial and nuclear markers revealed little divergence among the five populations analysed, suggesting the existence of panmictic populations of Indian Scad, *D. russelli* from Indian waters. The lack of significant genetic differentiation between populations as shown by AMOVA analysis ($F_{ST}$>0.05) suggests that *D. russelli* populations in Indian Ocean can be managed as a single stock. These findings contradict the previous study suggesting the presence of two distinct stocks of *D. russelli* along the east and west coast of India (Sen et al., 2011). The previous study was conducted based on truss morphometry over a small geographical area, with opportunistically collected individuals over a short period of time probably resulting in a potential artifact of population subdivision. Morphometric and meristic characters are particularly vulnerable to environmental influences and are considerably impacted once the species is exposed to environmental stressors. These environmental stressors act as a selection pressure affecting organisms' structure and function, reducing their Neo-Darwinian fitness (Calow 1989). The environmental conditions such as currents or water masses and various stressors including biotic and abiotic factors like food availability, salinity, temperature which are prone to variation both in time and space influencing the morphometry of species in each location at a specific time. Although it is widely acknowledged that the environment plays a role in shaping the phenotype, there are additional important factors influencing phenotypic variation which are being investigated at the molecular level (Burga and Lehner 2012). Thus, the variations reported by Sen et al. (2011) in the specimens from the Arabian sea and Bay of Bengal can be proposed as a consequence of phenotypic plasticity in response to temporal variations in environmental and oceanographic parameters.

One of the possible explanations for the observed lack of population subdivision in *D. russelli* samples include seasonal fluctuations in water circulation caused by the monsoon currents in the Indian Ocean. During the northeast monsoon, the flow of the upper ocean is directed westward towards the Arabian sea. The flow direction changes during the southwest monsoon, flowing eastward from Somalia towards the Bay of Bengal (Schott and McCreary 2001). This seasonal reversal of monsoon currents may homogenise *D. russelli* populations from various spawning grounds in Indian waters. Furthermore, the Indian Ocean is divided into two hemispheres by the equator, resulting in the known bioecological effects in the marine domain driven by the latitudes. In addition, this ocean is characterized by the westward South Equatorial Current (SEC) (Schott et al., 2009) and hydro chemical south Tropical Front which separate the two large oligotrophic areas – Indian Monsoon Gyre Province (MONS) in the

north and the Indian South Subtropical Gyre Province (ISSG) (Longhurst 1998). These physical and ecological separations however have little impact on determining the genetic structure of *D. russelli* as they appear to do in the Indo Malay Archipelago counterparts.

Analysis of mitochondrial *Cyt b* and *DrAldoB1* sequences indicated strong isolation between Indian Ocean and IMA with significant level of genetic differentiation. The central Indo-West Pacific region is geographically and hydrologically complex and possess the worlds' largest marine diversity hotspot (Mora et al., 2003). The region experienced major changes to its shorelines during the Pleistocene (Hewitt 2000; Voris 2000). The shallow seas of the Sunda shelf and Sahul shelf (Arafura Sea) were above the current sea level during the Pleistocene. Land barriers are also being built to separate the south China sea from the Indian Ocean at its southernmost point and from the sulu Sea to the east. The repeated fluctuation of the sea level associated with the ice ages may be the reason for the geographic isolation of several inland seas leading to the genetic differentiation of the species they harboured. This is believed to be the reason for the geographic discontinuity observed between the Indian and Pacific ocean of many marine species and also the greater species diversity observed in the Indo Malay region (Hewitt 2000). These environmental factors together with the emergence of land barriers to movement between ocean basins generally appear to be the strongest factor for the observed genetic heterogeneity of *D. russelli* populations between the Indian Ocean and IMA. This may also be responsible for shaping the modern genetic structure of many species that inhabit this region as we see it today (Avise 2000; Hewitt 2000; Bird et al., 2005, 2006).

Past geological and climatic events have undoubtedly played a major role in this inferred population structure. During the last interglacial period there were marine connections between the Indian Ocean and south China sea via the strait of Singapore (Bird et al., 2006). These connections may have allowed the *D. russelli*, essentially a pelagic species to freely migrate and expand its population across this region at that time. Estimation of the time since expansion of various lineages of *D. russelli* suggests the timing of these events are consistent with the cyclic sea level rises that occurred during the late Pleistocene between 1,600,000 to 10,000 years ago (Hanebuth et al., 2000). The land bridges that were exposed later as the sea level declined hampered the dispersal of *D. russelli*. Furthermore, the last glacial maximum (LGM) led to the final decline of sea levels to the present times which occurred between 30,000 to 19,000 years ago (Hanebuth et al., 2000; Lambeck et al., 2002). A substantial portion of Sunda and Sahul shelves were exposed as the sea level declined with glaciation up to 200m below their present level (Voris 2000). This process would have also impeded the dispersal of many

marine taxa. By this time the adjacent south China sea considerably shrank in size and transitioned into a semi closed marginal sea exposing a massive low gradient on the Sundaland carton (Hanebuth et al., 2000). In addition, around this time the modern Malayan Penisula, Borneo and Sumatra formed highlands to the south (Tjia and Liew 1996) adding yet another potential barrier to population migration. The co-occurrence of an IMA lineage and an IO lineage (clade 2 and clade 3), as well as their distinct geographic distributions, as seen in this study, can be attributed to the remnants of these earlier marine links and the emergence of geographic barriers brought about by ice ages associated with the Pleistocene events respectively. Such isolated marine populations experienced extensive inbreeding leading to the accumulation of genetic variation and structuring of their population at the genetic level.

## 4.6 Conclusion

The results of this study demonstrated that the Indian Ocean *D. russelli* constituted a single panmictic population and can be managed as a single stock. This study revealed two discrete populations of *D. russelli*, Indian Ocean populations and IMA populations. The inferred demographic history indicates that *D. russelli* populations may have experienced a potential population expansion during the Pleistocene. The subsequent appearance of a barrier to gene flow may have limited their ability to migrate between the Indian Ocean and the IMA as seen in the extant populations.

## 4.7 Future research directions

The genetic homogeneity in the Indian Ocean and the significant genetic differentiation detected between Indian Ocean and IMA needs to be corroborated by advanced genomic markers. The use of high-resolution genomic markers and single nucleotide polymorphism (SNPs) detected through genome scans may provide further insights into the genetic population stock structure of *D. russelli*. In addition, tagging programs and microchemical analyses of specific structures (eg: otolith) may also be helpful to devise management measures for *D. russelli* in the Indian Ocean. Finally, a comprehensive understanding of reproductive strategies, population dynamics in various regions, and spawning areas within the Indian Ocean is required to fully understand the nature and behaviour of its population.

# CHAPTER 5

## Chemical analysis of otolith for delineating stock structure in *D. russelli* along Indian coast.

## 5.1 Introduction

Otoliths are calcified structures exist in pairs in the inner ear cavity of teleost fishes. Though there are three pairs of otolith present, the term is usually referred by fishery biologist to represent the largest pair i.e., the Sagittae (Mathur et al., 2014). It has been proposed that the elemental composition of otolith can often serve as a natural marker or tag for group of fish inhabiting different environments (Campana 2005). Various environmental process that influence otolith composition have been extensively studied (Campana 1999; Secor and Rooker 2000; Elsdon and Gillanders 2003). The relationship between element and environmental process is often studied using element to calcium ratios (element/Ca) since the elements can replace Ca in $CaCO_3$ matrix and therefore the incorporation of elements into the matrix is depends on the Ca concentration (Elsdon et al., 2008). The use of otolith as a natural marker is largely depend upon its two characteristics features: First, otolith is metabolically inert meaning once deposited, the content does not resorb or dissolute (Campana and Neilson 1985). Next, the material deposited in the otolith is essentially a reflection of the physical and chemical environment of the water in which the fish inhabit (Gallahar and Kingsford 1992; Fowler et al., 1995). The uptake and incorporation of any given element or ion from the environment into the otolith matrix is multistep process involving the passage through more or less independent barriers (Campana 1999). So, we can't always say the elemental composition of otolith is always a reflection of the inhabiting environment. But the incorporation of at least some of the elements into the otolith is largely be influenced by environmental conditions and serve as environmentally influenced stock markers (Campana 2005) whereas, some other elements are under the influence of physiological (Campana 2005) and genetic (Campana 1999; Campana and Thorrold 2001; Elsdon et al., 2008; Sturrock et al., 2014) control. Nevertheless, even the elements that are under strict physiological control can be used to prove stock identity, as long as their concentration vary significantly among groups of fish (Campana 2005).

The chemical composition of otolith can be assayed by employing two methods: based on the whole dissolved otolith vs otolith core analysis (Campana 2005). As the otolith grows throughout the life of a fish, the first method integrates the entire lifetime of the fish species and thus serve as a natural tag for fish groups experiencing different environmental conditions. In contrast analysing the otolith core is essentially used as a direct measure of stock or nursery origin. These two methods allowed the application of otolith chemical composition for a wide range of studies including identification of natal origin or nursery grounds (Gillanders 2002;

Clarke et al., 2009; Loher et al., 2021), classifying adults based on their area of origin (Reis-Santos et al., 2013; Artetxe-Arrate et al., 2021; Russell et al., 2021), migration pattern (Hamer et al., 2006; Fairclough et al., 2011; Albertsen et al., 2021; Bounket et al., 2021), discriminating stock and determining population structure (Arkhipkin et al., 2009; Tanner et al., 2012; Artetxe-Arrate et al., 2021; de Souza Corrêa et al., 2022).

The advantage of using the chemical signatures of otoliths is that they can reveal the spatiotemporal environmental gradient that the fish experience throughout their lives (Soeth et al., 2019). The elemental chemistry of the otolith has been successfully used to elucidate the population structure of several fish species, even in systems with high gene flow where environmental heterogeneity exists (Bradbury et al., 2008; Smith and Campana 2010; Correia et al., 2012) and thus provide useful information about population dynamics (Ferreira et al., 2019; Soeth et al., 2019), movement patterns and habitat connectivity (Adelir-Alves et al., 2018). Recently, several studies have been conducted that coupled otolith chemistry and genetic analyses and have shown sometimes contradictory (Collins et al., 2013) and complementary (Feyrer et al., 2007) results revealing natal origin of individuals, habitat use and regional level genetic structure. In many cases, the chemical analysis of otolith has resolved several uncertainties on natal habitats and stock structure in both marine and freshwater environments, that genetic markers were unable to address (Thorrold et al., 1998; Campana et al., 2000; Milton and Chenery 2001). This was made possible by the fact that, in contrast to population divergence detected by genetic markers, which usually requires a longer time scale, otolith microchemistry can be used to identify specific traits or events that have occurred within the time scale of an individual's lifetime. Therefore, it is necessary to combine multiple methodological approaches to fully comprehend the spatial ecology and management requirements of a fish species and to influence local management practices.

## 5.2 Review of literature

Preliminary studies carried out as early as 1967 suggested the constituents and trace elements embedded in otoliths, vertebrae and scales could be useful in delineating data on population structure and movement pattern of fish species (Fishery Agency of Japan 1967). In the early 1970s, a number of attempts have been carried out to test this assumption of whether the constituents of calcified structure can resolve stock or sub population identity in fishes (Calaprice 1971; Bagenal et al., 1973) by employing various analytical techniques. From the mid-1990s there was steady increase in the studies on otolith chemical analysis (Gunn et al.,

1992; Secor et al., 1995; Otake and Uchida 1998) which were on peak during 2010 (Cuveliers et al., 2010; Longmore et al., 2010; Pangle et al., 2010; Zeigler and Whitledge 2010). These studies can be categorized according to their methodological approach either by using the whole otolith or targeted to specific points in the otolith (e.g., core). Determining the methodological approach is the crucial step in the otolith chemical studies as thus largely determines the ecological questions and the spatio-temporal scope that can be addressed. The first studies that used otolith chemical analysis to distinguish stock used the whole otolith analysis employing X-ray spectrometry (Calaprice 1971) and solution based Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) (Edmonds et al., 1991). Many studies have then employed the use of whole otolith chemistry to identify stock structure of species successfully discriminate different stocks (Campana et al., 2000; Jónsdóttir et al., 2006; Swan et al., 2006; Correia et al., 2012).

It has been proved based on vast research that the elements which can be used as environmentally influenced natural markers for stock identity include Strontium (Sr), Barium (Ba), Manganese (Mn), Iron (Fe), Lead (Pb), Lithium (Li), Magnesium (Mg), Copper (Cu) and Nickel (Ni) ( Fowler et al., 1995; Farrell and Campana 1996; Dove 1997; Geffen et al., 1998; Campana 1999; Bath et al., 2000; Milton and Chenery 2001). In addition, many studies have employed the isotopes of certain elements as natural tags for delineating population structure of fish (Kennedy et al., 1997; Spencer et al., 2000; Thorrold et al., 2001; Weber et al., 2002). Other researchers have investigated ways to overcome the lack of heterogeneity in the marine environment by utilising various elements, such as Bromine (Br) and Selenium (Se), (Limburg et al., 2010; Lochet et al., 2010) and rare earth elements, such as Scandium (Sc), Lanthanum (La), and Dysprosium (Dy) (Dorval et al., 2005; Lara et al., 2008). Recently, Isoscapes (spatial maps of expected isotopic variation) and ecogeochemical techniques have demonstrated significantly greater regional variability than previously believed, allowing them to be used as the foundation for investigations on stock delineation and migration patterns (Trueman et al., 2012; McMahon et al., 2013; Darnaude et al., 2014).

Ther is no a priori reason for considering the elemental fingerprint can be function as an indicator for population identity, even when there are no genetic differences. Besides, just as the environment at a particular location wouldn't stay steady over time, there is no reason to believe that the otolith fingerprint of fish will be consistent over a long period of time. Nonetheless, because otolith grows continually during the life of the fish, the elemental fingerprint of the entire dissolved otolith integrates throughout the course of its lifetime and

can be used to differentiate between fish that have been exposed to various environmental conditions (Edmonds et al., 1989, 1991, 1992; Campana et al., 1995; Bronte et al., 1996; Gillanders and Kingsford 1996; Kennedy et al., 1997; Thorrold et al., 1998). Otolith elemental composition may therefore function as a stock identity marker as long as fish population or stocks inhabit different environmental conditions. Whole otolith fingerprints, on the other hand, may be applied more robustly to tracking stock migrations or stock mixing in natural populations. In these scenarios, the fingerprints serve as biological tracers or natural tags for pre-defined fish groups over brief time periods (Campana et al., 1995; Gillanders and Kingsford 1996; Kennedy et al., 1997). Using otolith elemental fingerprint as a natural marker would exploit the fact that freshly added otolith material contributes to only a small proportion of the total otolith mass. Therefore, it is reasonable to assume that the changes in the environment and otolith growth during the (brief) period of migration or mixing will have very little impact on the fingerprint (Campana et al., 2000). In principle, fish should be able to identified as belonging to their source group until their elemental composition has been changed by subsequent otolith growth, provided that all possible source group are defined before the time of mixing or migration ( Campana et al., 2000; Campana 2005).

## 5.3 Materials and Methods

### 5.3.1 Study area and sample collection

In 2023, we collected *D. russelli* from four locations along the Indian coast from January to March: two locations along east coast (Digha and Chennai) and two locations along west coast (Veraval and Cochin) of India (Figure 5.1). The locations were chosen to align with the previous genetic analysis conducted, facilitating a comparison to corroborate the genetic findings. Twenty-five specimens were collected from each site using trawl and purse seines operating near the coast. Whole fish were preserved frozen in the field and returned to the laboratory for processing. The length (TL) (cm) of fish was recorded before preparing the otolith for subsequent analysis.

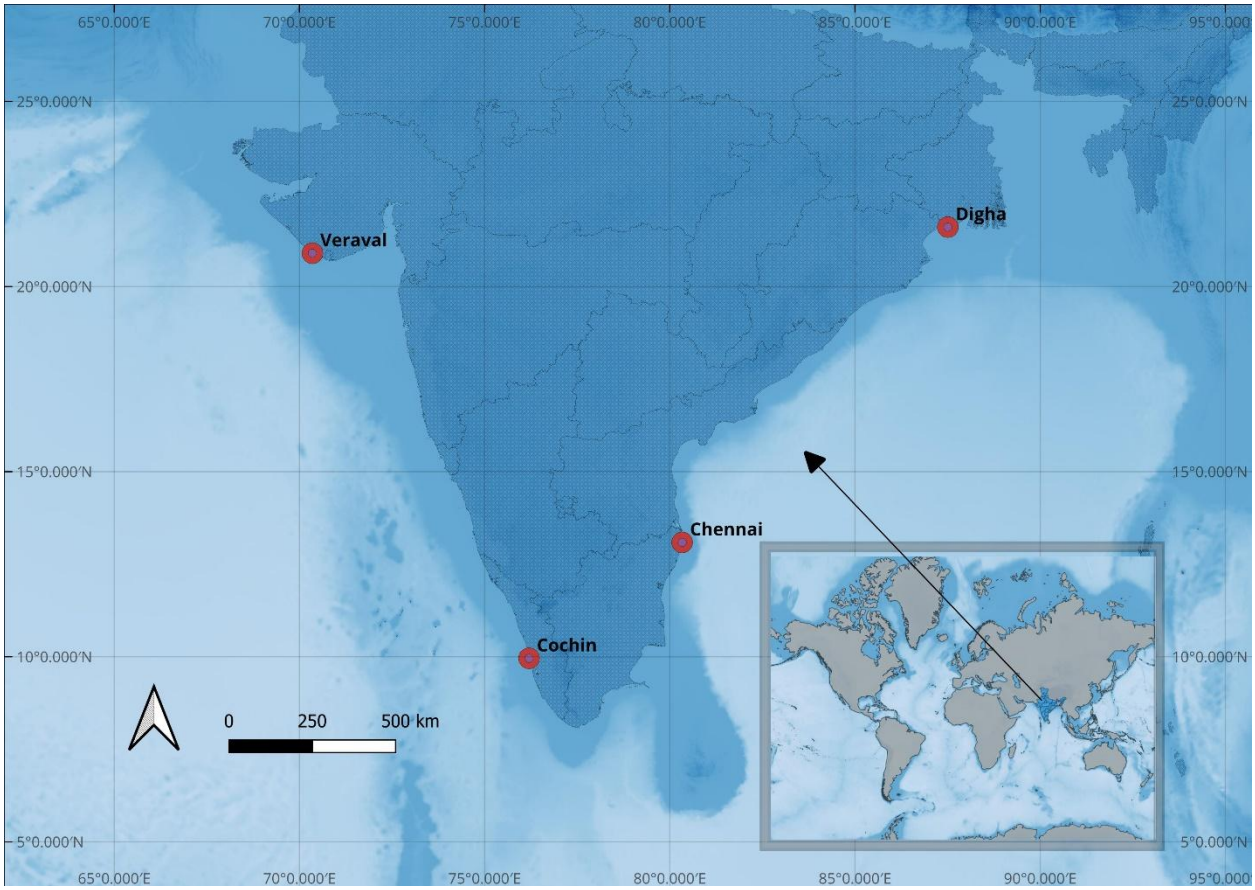


Figure 5.1 Map indicating sampling locations of *D. russelli* along Indian Ocean.

### 5.3.2 Otolith preparation

A pair of sagittal otoliths were removed by open the hatch method (Secor et al., 1992) (Figure 5.2) from the 25 specimens from each study site. All adhering tissues were removed using an acid washed nylon brush and forceps. The otoliths are then decontaminated by rinsing with Milli-Q water and then completely air dried. Following decontamination, the otolith was stored until processing in vials which are previously washed with dilute nitric acid and ultra distilled water and oven dried. Before storage the otolith weight (g) was recorded for each individual.

### 5.3.3 Otolith chemical analysis

A pair of sagittal otoliths from each individual are first digested with 4 ml of 1-2% trace metal grade nitric acid (TraceMetal™ Grade, Fisher Scientific) and 1 ml of 30-32% $H_2O_2$ (Optima, Fisher Scientific) using Milestone Start D microwave extraction system (Milistone s.r.l., Sorisole, Italy), equipped with easy CONTROL software and HPR 1000/10S high pressure segmented rotor at a pressure of 400 psi and power of 1200 W. The conditions for sample digestion were set as follows: ramp upto 180°C for 20 minutes; hold at 180 °C for 20 minutes

and cooled down for 10 minutes at room temperature. Prior to analysis, the digested samples were filtered using a 0.45 micron syringe filter and diluted to known volume with ultra-pure water. Microwave condition and digestion procedures were followed according to Milestone Cookbook Digestion Rev. 03_04. Temperature and pressure sensors were used to monitor digestion conditions. The elemental analysis of *D. russelli* otolith samples were carried out using Inductivity Coupled Plasma–Optical Emission Spectrometer (ICP-OES) (iCAP 6300 Duo, Thermo fisher Scientific, Cambridge, England) with dual configuration (axial and radial) and iTEVA operational software (version 2.8.0.97). The instrumental conditions used were: optics temperature 38°C, camera temperature of -44°C; Nebulizer (MiraMist Cyclonic Chamber) main argon flow rate of 15 L per min, auxiliary argon flow rate of 0.5 L per min, gas flow of 0.5 L per min and maximum integration time of 30 sec. ICP multi-element standard solution (CentiPUR, Merck-Supelco®, Mumbai, India) was used for the preparation of calibration solutions. Five calibration levels of multi-element standard ranging from 0.2 to 2 mg/kg were prepared by dilution with 1% HNO3. Test value specified for the acid are ≤ 1 µg/Kg in case of Ca, Fe, K, Mg and Na, whereas ≤ 0.1 µg/Kg for Ba and Sr, ≤ 0.5 µg/Kg for Zn. Reagent blanks were analysed along with digested samples to confirm any presence of contamination. The method for analysis of metals in fish using ICP-OES is validated as per requirement of AOAC Guidelines for Standard Method Performance Requirements. The samples were analysed for Barium (Ba), Iron (Fe), Magnesium (Mg), Potassium (K), Sodium (Na), strontium (Sr), Zinc (Zn) and reported as ratios to calcium (Ca). The mean concentration (ppm) of each element from the four-sampling location detected via ICP-OES is shown in Table 5.1.

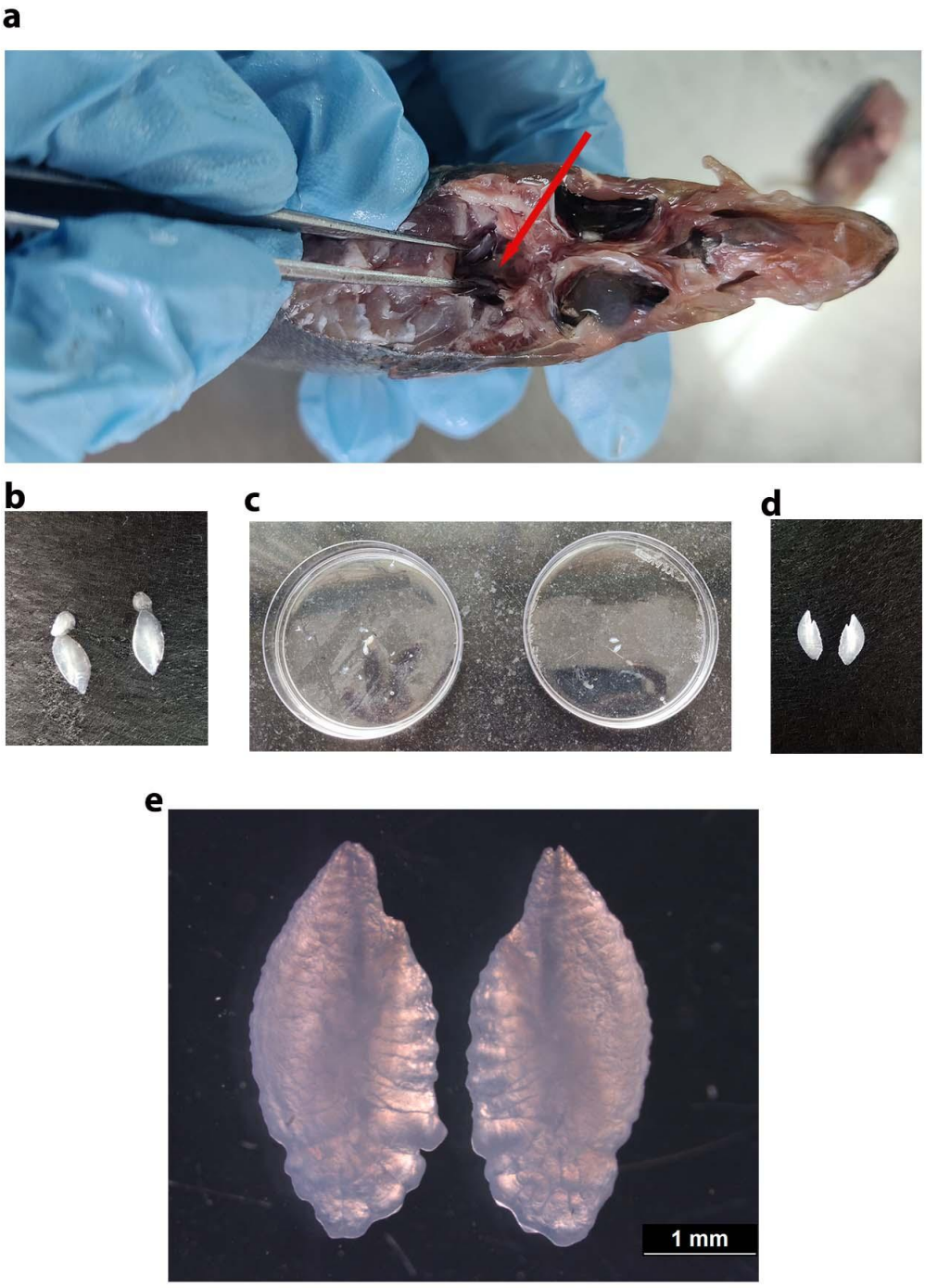


Figure 5.2 Removal and preparation of otolith for elemental analysis (a) Removal of otolith by open the hatch method; red arrow indicates the location of the otolith. (b) All the three pairs of otolith and adherent tissue. (c) Washing and (d) cleaning of sagittal otolith (e) Stereo zoom image of a pair of sagittal otoliths from *D. russelli*.

Table 5.1 Summary table of the ICP-OES data (concentration in ppm) for all four study **sites.**

| Sl.No | Element | Chennai | Cochin | Digha | Veraval |
|---|---|---|---|---|---|
| | | Samples (n=25) | Samples (n=25) | Samples (n=25) | Samples (n=25) |
| | | Average ± SD | Average ± SD | Average ± SD | Average ± SD |
| 1 | Ba | 25.973 ± 7.70 | 14.298 ± 14.01 | 57.680 ±35.38 | 17.999 ± 18.23 |
| 2 | Ca | 2,79,681 ± 54493 | 2,20,376±202507 | 2,37,155 ± 11309 | 2,51,396 ± 86674 |
| 3 | Fe | 389.684± 313.57 | 361.852± 192.34 | 1168.902±715.59 | 64.1597± 33.70 |
| 4 | K | 296.376 ± 147.53 | 347.148± 143.32 | 498.626 ± 11.26 | 84.9862 ± 45.00 |
| 5 | Mg | 204.852 ± 87.85 | 81.002 ± 48.28 | 37.753 ± 302.09 | 60.652 ± 40.16 |
| 6 | Na | 2988.633 ±1457.72 | 1657.52 ± 582.61 | 3730.206 ± 406.54 | 1574.301 ± 1010.53 |
| 7 | Sr | 2162.252 ± 855.86 | 2435.88 ± 199.89 | 2407.852 ± 155.54 | 1744.03 ± 990.05 |
| 8 | Zn | 87.0468 ± 209.29 | 132.423 ± 63.25 | 120.183 ± 67.14 | 36.645 ± 117.20 |

### 5.3.4 Statistical analyses

The data collected on element/Ca ratios in otoliths from different locations/regions were subjected to one-way Analysis of Variance (ANOVA) using PROC GLM of SAS software (Ver. 9.3) to find the significant difference among the locations/regions. Further, a multiple comparison among the locations/regions were made using Tukey's HSD test. The significant difference between locations/regions were depicted by different alphabets. The data were transformed by using square root transformation to make it amenable to ANOVA. The significance was assessed at $p<0.05$. A Multivariate ANOVA (MANOVA) was performed to assess the significance among the study sites/regions while considering all the element/Ca ratios simultaneously based on four statistical tests viz., Wilk's Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest Root. Spearman's rank correlation coefficient was worked out to find the degree of linear relationship of the element/Ca ratios with the total length (TL) and otolith weight (W). Linear Discriminant Analysis (LDA) was performed using all 100 observations to determine if study sites could be discriminated based upon the elemental

composition of otoliths using the R software (R Core Team 2021) after standardizing the variables (element/Ca ratios). An LDA plot was also drawn using the one the first two linear discriminant function scores to depict the classification of samples.

## 5.4 Results

### 5.4.1 Single elemental analysis

We conducted the statistical analysis by grouping the data by coast (East and West) and by location (Chennai, Cochin, Digha and Veraval) to test the differences in elemental concentration at a finer geographical scale. All element/Ca ratios (Ba/Ca, Fe/Ca, K/Ca, Mg/Ca, Na/Ca, Sr/Ca, Zn/Ca) for the otolith differed significantly between the four sampling sites (one way ANOVA, $P<0.05$) (Figure 5.3). It has been demonstrated that region explained the most variation in K/Ca and Fe/Ca. On comparison of element/Ca ratios across location revealed that otoliths from Digha had significantly higher ratios for Ba/Ca, Fe/Ca, K/Ca, Mg/Ca and Na/Ca (Tukey's, $p<0.05$) (Figure 5.3 a, b, c, d and e) whereas otoliths from Cochin revealed significantly higher Sr/Ca and Zn/ Ca ratios (Tukey's, $p<0.05$) (Figure 5.3 f and g). All element/Ca ratios were significantly lower in the Veraval otolith (Tukey's, $p<0.05$) (Figure 5.3). Further comparative analysis showed that, Ba/Ca, Mg/Ca and Na/Ca ratios were significantly higher in Chennai otoliths than in Cochin (Tukey's, $p<0.05$) (Figure 5.3 a, d, e) and Fe/Ca ratios were significantly higher in Cochin otoliths than in Chennai (Tukey's, $p<0.05$) (Figure 5.3 b). Furthermore, the Digha otolith demonstrated significantly higher Sr/Ca and Zn/Ca ratios (Tukey's, $p<0.05$) compared to the Chennai otolith (Figure 5.3 f and g).

When we analysed the data by coast (east and west), it has been shown that, all element/Ca ratios were found to be significantly different between the coast (one way ANOVA, $P<0.0001$) with the exception of Sr/Ca ($p=0.380$) and Zn/Ca ($p=0.515$) (Figure 5.4 f and g). Ba/Ca, Fe/Ca, K/Ca, Mg/Ca and Na/Ca ratios were significantly higher on the east coast (Tukey's, $p<0.05$) (Figure 5.4 a, b, c, d and e). Region explained the most variation in Mg/Ca and Na/ Ca.

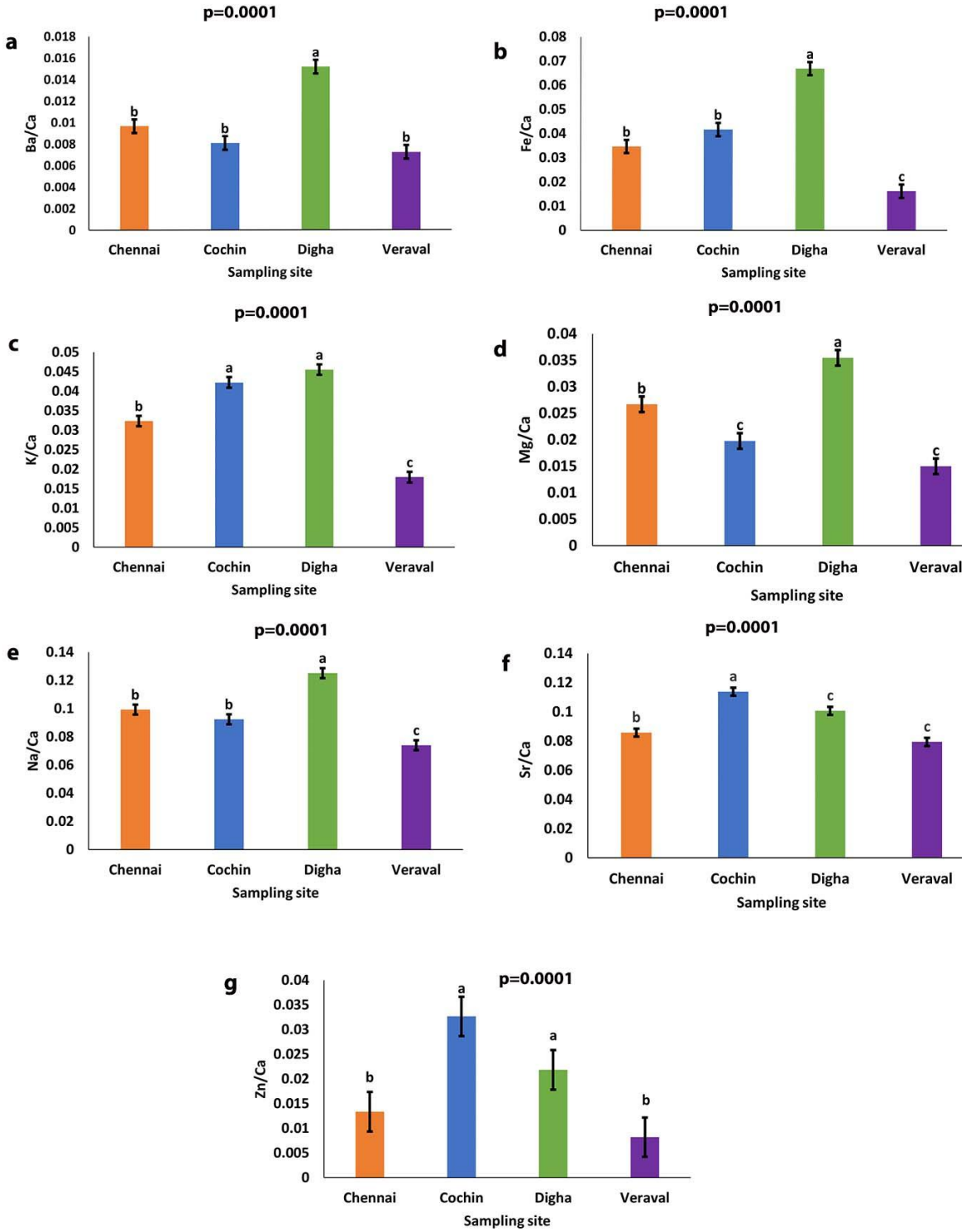


Figure 5.3 Location wise comparison of element/Ca ratios from the whole otolith of *D. russelli* from Cochin, Chennai, Digha and Veraval. (a) Ba/Ca ratios. (b) Fe/Ca ratios. (c) K/Ca ratios (d) Mg/Ca ratios. (e) Na/ Ca ratios. (f) Sr/Ca ratios. (g) Zn/Ca ratios. Different letters above the boxes indicate significant differences between locations.

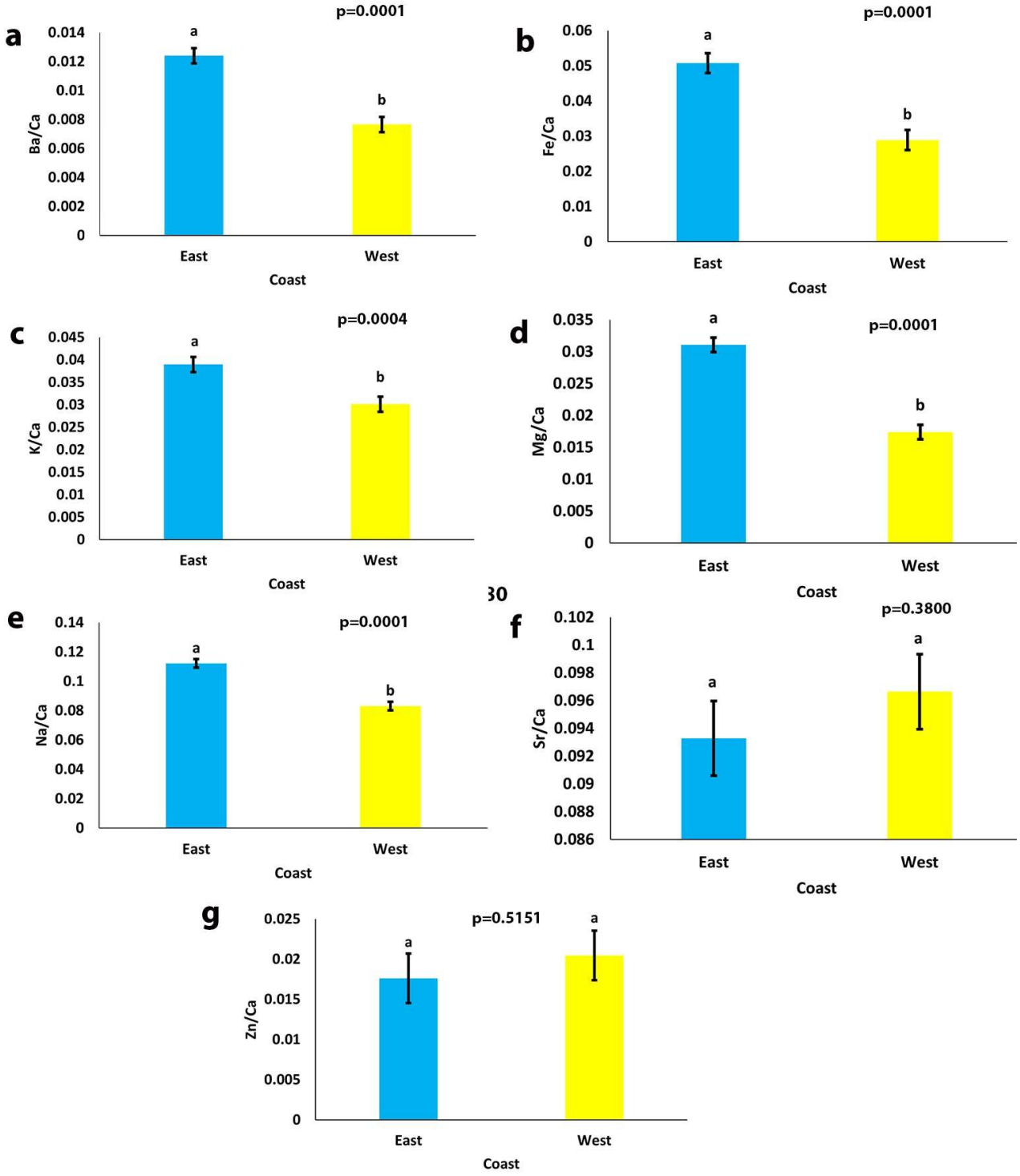


Figure 5.4 Coast wise comparison of element/Ca ratios from the whole otolith of *D. russelli* from east and west coast of Indian Ocean. (a) Ba/Ca ratios. (b) Fe/Ca ratios. (c) K/Ca ratios (d) Mg/Ca ratios. (e) Na/ Ca ratios. (f) Sr/Ca ratios. (g) Zn/Ca ratios. Different letters above the boxes indicate significant differences between coast.

### 5.4.2 Multi elemental analysis

Multivariate analysis (MANOVA) based on otolith chemistry demonstrated significant differences between all sampling sites (Wilk's Lambda, $p<0.0001$; Pillai's Trace, $p<0.0001$; Hotelling-Lawley Trace, $p<0.0001$; Roy's Greatest Root, $p<0.0001$) (Table 5.2). It also revealed significant differences between the east and west coast of India (Wilk's Lambda, $p<0.0001$; Pillai's Trace, $p<0.0001$; Hotelling-Lawley Trace, $p<0.0001$; Roy's Greatest Root, $p<0.0001$) (Table 5.3).

Table 5.2 MANOVA test criteria and F approximations for overall location effect

| Statistic | Value | F Value | P value |
|---|---|---|---|
| **Wilks' Lambda** | 0.02908255 | 22.50 | <.0001 |
| **Pillai's Trace** | 1.76873808 | 14.37 | <.0001 |
| **Hotelling-Lawley Trace** | 9.08194043 | 29.24 | <.0001 |
| **Roy's Greatest Root** | 4.84647871 | 48.46 | <.0001 |

Table 5.3 MANOVA test criteria and F approximations for overall coast effect

| Statistic | Value | F Value | P value |
|---|---|---|---|
| **Wilks' Lambda** | 0.30910326 | 22.35 | <.0001 |
| **Pillai's Trace** | 0.69089674 | 22.35 | <.0001 |
| **Hotelling-Lawley Trace** | 2.23516487 | 22.35 | <.0001 |
| **Roy's Greatest Root** | 2.23516487 | 22.35 | <.0001 |

We found no correlation between total length (TL) and otolith weight (W) on element/Ca ratios. The F approximations for all of the multivariate statistics in the Linear Discriminant Analysis (LDA) were significant with p values <0.0001, indicating that it is possible to distinguish between 4 sites based upon *D. russelli* otolith elemental composition. The first two axes of the LDA explained 97% of the variability, with the first axis being the more representative (60%) (Figure 5.5, Table 5.4). Based on the coefficient of linear discriminants, the most relevant variable for classification were Na/Ca (-2.46) and Sr/Ca (1.70) for the first function and Na (-0.49) and K/Ca (1.39) for the second function (Table 5.4).

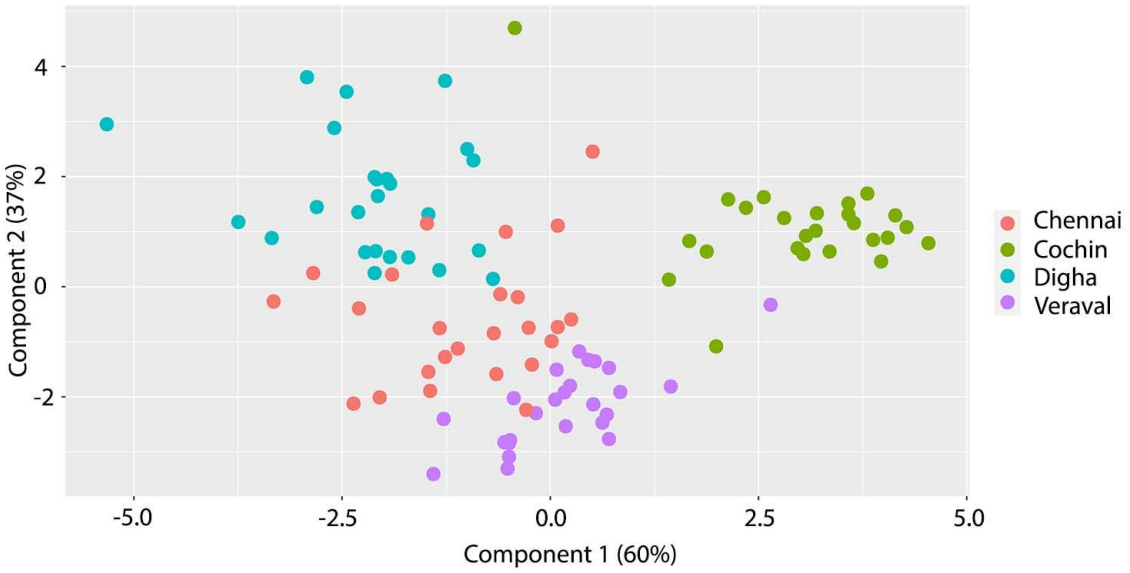


Figure 5.5 Plot of individual fish labelled by site, using variates derived from linear discriminant analysis of elemental ratios. Colored circles represent different locations (refer to legend).

Table 5.4 Standardised coefficients and percent dispersion explained for the first two discriminant components (LD1 and LD2) in the analysis of otolith chemical signatures of *D. russelli*.

| | **LD1** | **LD2** |
|---|---|---|
| Ba/Ca | -0.50864830 | -0.02453792 |
| Fe/Ca | -0.01063478 | 0.68050986 |
| K/Ca | 0.55108912 | 1.38804510 |
| Mg/Ca | -0.04406014 | -0.36318700 |
| Na/Ca | -2.45794559 | -0.48450698 |
| Sr/Ca | 1.69282975 | 0.61540761 |
| Zn/Ca | 0.06171334 | 0.08510018 |
| Dispersion (%) | 60 | 37 |

## 5.5 Discussion

This study evaluated for the first time the chemical analysis of otolith as a potential tool for distinguishing *D. russelli* stocks in the Indian Ocean. The chemical analyses of the otolith indicate significant spatial differences among the four population collected across the Indian Ocean. The observed differences in the whole otolith chemistry among sampling sites suggested environmental heterogeneity of the elements examined between these sites. The result of this spatial difference of *D. russelli* observed in this study contrasts with the panmictic nature of its population observed across the Indian Ocean based on genetic analysis. However, this confirms the previous reports of different stocks of this species along the east and west coasts of India based on morphometric data (Sen et al., 2011). *D. russelli* has been reported to be essentially a pelagic species that undertakes long distance migrations (Hardenberg 2016). Despite the morphometric (Sen et al., 2011) and otolith chemical (this study) evidence of distinct stock reported for this species, there may be sufficient genetic mixing may exist between its populations to prevent population differentiation at the genetic level. The species is reported to have an extended spawning season that overlaps along different coasts of India (Sreenivasan 1981; Murty 1991; Reuben et al., 1992; Raje 1997; Balasubrahmanian and Natarajan 2000; Manojkumar 2007; Poojary et al., 2015), suggesting that it is a continuous breeder that prefers to spawn when conditions are favourable. This means that the species would have multiple opportunities to spawn and reproduce throughout its life. If the degree of mixing continues into the breeding season, it would be no surprise that *D. russelli* are effectively panmictic within the study region.

The chemical signature in the otolith of individuals or groups of fish inhabiting in different environments can be substantially different depending on various factors including how long they remain in a particular environment before being caught to integrate a detectable chemical tag, the differences in the physico-chemical properties of the inhabiting environment and the elements selected for analysis (Elsdon et al., 2008). However, the distance between locations however had little impact on the degree of variation in otolith chemistry as it reflects only on the variation in environmental properties and scales at which it varies (Elsdon and Gillanders 2003). Sometimes, the groups of fish that have separated in different locations during the same life history stage or only a short life history stage during the whole otolith tag may also chemically distinguishable from one another (Elsdon and Gillanders 2003; Jónsdóttir et al., 2006).

The existence of different stocks observed for the species in the present study can be explained by the climatic phenomena and process associated with different part of the Indian Ocean. The southern Indian Ocean is characterized by a westward flowing south equatorial current (SEC), and is primarily supplied by the ITF, which later split into northward and southward branches forming a major conduit for tropical-subtropical exchange and supply water for East African Coastal Current (EACC) (de Ruijter et al., 2002). The eastern Indian Ocean is marked by the existence of an intensified zonal eastward flow which expands latitudinally to the east. In addition, East Gyral Current (EGC), which is the eastward geostrophic flow across the ITF regime supplied from the north (Meyers 1996). The northern Indian Ocean is characterized by the northward Somali current supplied by SEC and EACC during the summer monsoon (Vecchi et al., 2004). The Somali current forms various cells and gyres as it proceeds: Southern Gyre as it flows across the equator, Great whirl in the north, Socotra Eddy in the Northeast. It also forms a cold upwelling wedge after crossing the equator. In the winter monsoon the Somali current flows southward meet the EACC, supplying water for the eastward flowing South Equatorial Counter current (SECC) (Song et al., 2004). These inter oceanic flows and upwelling wedges associated with these currents may have an impact on the surface wind stress and heat fluxes (McCreary et al., 2007), which may impact the composition of water chemistry at the sampling sites as there are reports which states the influence of these factors in determining the water chemistry at a region (Elsdon and Gillanders 2003).

In general, migratory fishes are considered to have reduced population structure in marine environments because marine environments present fewer barriers to dispersal and the species can therefore undertake long migrations (Ward et al., 1994). Many studies support this assumption and found low population structure in migratory species (McKeown et al., 2015). In contrast, there are studies which reported stock structure in migratory species using otolith chemical analysis (Svedäng et al., 2010; Walther and Limburg 2012; Amano et al., 2015; Fortunato et al., 2017; Moreira et al., 2018). Still some other studies have employed both otolith and genetic analysis and revealed corroborating (McKeown et al., 2015) and contrasting (Collins et al., 2013) results regarding the stock structure of marine species. The result of the present study based on otolith chemical analysis as opposed to genetic analysis can be attributed to the ability of different markers to reveal different levels of connectivity. Thus, the observed lack of genetic structure for *D. russelli* is not necessarily inconsistent with our finding from otolith chemical analysis, but rather highlights the need to consider both ecological (otolith chemistry) and evolutionary connectivity (molecular genetic markers) to understand

the systems with extremely low levels of population structure. Previous study based on truss morphometric analysis also revealed separate stocks of the species on the east and west coast of India (Sen et al., 2011) confirming our finding that environmental variation can influence the distribution patterns of the species.

Even though we observed statistically significant evidence for considering the four sampling locations as different population units, we cannot be conclusive about the stock structure across east and west coast as reported by Sen et al. (2011). The LDA plot obtained in the study somehow explained the discrimination between sampling sites, there are, albeit low overlapping of individuals can be seen, suggesting a more or less pelagic dispersal pattern of *D. russelli* consistent with its observed genetic homogeneity. All elements that show significant differences across the coast had a higher proportion on the east coast than on the west coast. This can be attributed to the Digha otolith, which showed a higher percentage of variation in most elements.

It has been evident based on previous research that the element Sr is significantly influenced by its ambient concentration (Moreira et al., 2018). In addition, it is known that there is a positive relationship between Sr/Ca and water salinity (Bath et al., 2000; Secor and Rooker 2000; Elsdon and Gillanders 2003). So the higher Sr/Ca ratio observed in the Digha otolith consistent with the Cochin otolith can therefore be attributed to the southwest monsoon currents bringing saltier Arabian Sea water to the Bay of Bengal (Elsdon and Gillanders 2003). The upwelling process and flow of ocean currents are sometimes linked to water chemistry and Ba appears to be a reliable indicator of these changes. The intensified zonal eastward flow (Song et al., 2004), the eastward geostrophic flow, EGC (Meyers 1996) and the Socotra Eddy Cell of the Somali current (Song et al., 2004) in the northeast could explain these results. Moreover, previous studies reporting a positive relationship between Ba/Ca and salinity (Miller 2011) were consistent with the Ba results in the present study. In addition, there are reports of a positive relationship between Sr and Ba indicating that increased Sr concentration can facilitate the uptake of Ba into the fish otolith (de Vries et al., 2005). Further, there is also a positive correlation between Mg and salinity (Nelson and Powers 2020) confirming our findings. There is no proof that the physiochemical characteristics of the surroundings affect the integration of Fe, despite a study that found no correlation between the temperature and salinity of the environment and the uptake of Fe. (Kakuta et al., 1997). Zn/ Ca is reported to be inversely proportional to salinity (Kakuta et al., 1997). Although the waters of the Arabian Sea are salty, the westward flow of northeast monsoon current carries the fresh waters of the Bay of Bengal

into the Arabian sea making it less salty. This explains the higher Zn/Ca ratios observed in the Cochin otolith in the present study. In addition, Wyrtki Jets (WJs), which occur only in the Indian Ocean, transport warm upper layer water eastward, causing sea level and mixed layer thickness to rise in the east and decrease in the west (Schott et al., 2009). This remarkable Indian Ocean phenomenon could be responsible for the exceptionally low concentration of all element/Ca ratios in the Veraval otolith and also for the exceptionally higher concentration of elements on the east coast, since almost all of the elements we discussed have a positive correlation with temperature (Bath et al., 2000; Secor and Rooker 2000; Miller 2011; Nelson and Powers 2020;). The remaining elements K and Na are reported to be essential for cellular processes and have an additional physiological response to their regulation in addition to environment (Thresher et al., 1995).

Our results provide new information and independent confirmation of previous studies on the population structure of the *D. russelli* in the Indian Ocean, based on morphometric analyses and information that contradicts the previous suggestion based on genetic analyses that *D. russelli* is panmictic. This basic information on ecology and population structure is crucial as a basis for formulating effective management and conservation strategies for the species. Future studies using data collected along a complete ontogenetic transect of the otolith is necessary to examine whether individuals migrate to the same location for feeding and breeding throughout their lives to distinguish between the mechanism of genetic homogenization and the ecological differentiation. In addition, further sampling during the species' breeding season may provide sufficient information about whether the population structure is present. If individuals return to the same location to breed every year, there should be a strong population structure at the genetic level, which unfortunately is not the case for *D. russelli*. In addition, sampling before and after the breeding season can also help examine the structure of the population at a finer scale.

## 5.6 Conclusion

The present study reported significant differences in elemental composition among the four sampling sites. Five of the seven element/Ca ratios contributed to the differentiation among coast with east represents the largest contribution of the overall elemental composition. The near-surface circulation and the upwelling in different regions of the Indian Ocean may contribute to the unique elemental composition observed in particular location. The finding supports the existence of different management units for the species. The study also highlights

the discriminatory capacity of various methods and the need to use multiple approaches to determine the spatial ecology of highly migratory fish species. The contrasting results obtained here based on genetic and chemical approaches indicated that both provide information on different time scales: ecological for otolith chemistry and evolutionary for genetics.

## 5.7 Future research directions

The ecological differentiation observed in the present study is needed to be further analyzed based on data collected along a complete ontogenetic transect of the otolith to examine whether individuals migrate to the same location for feeding and breeding throughout their lives to distinguish between the mechanism of genetic homogenization and the ecological differentiation. In addition, further sampling during the species' breeding season may provide sufficient information about whether the population structure is present. If individuals return to the same location to breed every year, there should be a strong population structure at the genetic level, which unfortunately is not the case for *D. russelli*. In addition, sampling before and after the breeding season can also help examine the structure of the population at a finer scale.

# CHAPTER 6

## Final conclusions, Significance and Societal contributions of the study

## 6.1 Final conclusions

The present study aimed to characterize the complete mitochondrial genome of *Decapterus russelli* and to conduct a comparative phylogenetic and evolutionary analysis within the family *Carangidae*. Additionally, the study explored the population structure of *D. russelli* using both genetic markers and otolith chemical analysis.

Key conclusions from this research are as follows:

**6.1.1 Mitochondrial genome characterization and phylogenetic insights**:

- The complete mitochondrial genome of *D. russelli* was characterized, with comparative analyses within *Carangidae* providing insights into genomic variability and the evolutionary history of the family.
- The Ka/Ks and P genetic distance analysis have shown that the PCGs of Carangidae are under strong purifying selection.
- The carangid tRNA was structurally different in both the stem and loop, with the exception of tRNA-Trp, and tRNA-Leu (TAG). These structural features resulted in mismatched or wobble base pairs in tRNAs.
- The high rate of Watson-Crick and Wobble base pairing in the stem regions of tRNA suggested their role in stem structure maintenance.
- The classical cloverleaf secondary structure was retained in all tRNAs except tRNA-Ser (GCT).
- The presence of an mTERF binding site and a very high evolutionary conservation of its sequence suggest the functional significance of these sites.
- The high rate of sequence divergence in the flanking regions of conserved sequence motifs indicated the absence of typical mitochondrial control region coding constraints.
- The presence of evolutionarily conserved secondary structures of the conserved sequence motifs despite sequence divergence revealed the effect of selective constraints on their maintenance.
- The length variations in protein-coding, tRNA, and rRNA genes among Carangidae were negligible.
- The phylogeny inferred from 13 gene concatenated data set resolved the monophyletic lineage of each subfamily and robustly supported the grouping (Trachinotinae+(Caranginae+Naucrautinae).
- The speciation of the genus *Decapterus* occurred before the separation of Tethys Sea and *D. russelli* diverged before the mid-Pliocene warm period.

### 6.1.2 Genetic structure and connectivity:

- The present study found that Indian Ocean *D. russelli* populations collected from five different locations of Indian Ocean are essentially panmictic based on mitochondrial (*Cyt b*) and nuclear (*DrAldoB1*) markers.
- AMOVA analysis undertaken with mitochondrial and nuclear markers shown lack of significant genetic structure in *D. russelli* among the five-population from the Indian Ocean analyzed.
- The haplotype network diagram produced a reticulation of 38 haplotypes.
- Phylogenetic analysis based on mitochondrial and nuclear markers revealed no obvious phylogeographic pattern separating the 5 samples of *D. russelli*.
- Based on comparative analyses, we observed the presence of possible genetic break between the populations of *D. russelli* from the Indian Ocean and the Indo Malay Archipelago.
- The genetic diversity or distance analysis have shown significant differences in the $F_{ST}$ values ($P<0.05$) between the populations from the IMA and Indian Ocean.
- The phylogenetic tree and haplotype network diagram revealed distinct mitochondrial lineages corresponding to IMA and Indian Ocean populations.
- Negative values of Tajimas' D and Fu's Fs, harpending raggedness index and mismatch analysis indicated a recent historical directional selection (selective sweep) or population expansion of *D. russelli* population.
- The genetic homogeneity observed for this species from Indian Ocean is likely due to the species' migratory nature and oceanic currents and the genetic discontinuity observed between populations in the Indian Ocean and the Indo-Malay Archipelago, possibly resulting from historical sea-level changes during the late Pleistocene, which contributed to genetic divergence between these regions.
- The results suggest *D. russelli* from the Indian Ocean can be managed as a single stock and the populations from Indian Ocean and IMA can be treated as separate management units.

### 6.1.3 Environmental differentiation and spatial structure:

- Otolith chemical analysis revealed significant spatial variation in elemental compositions along the Indian coast, reflecting ecological differentiation at a regional level.

- This spatial structuring aligns with environmental variation, driven by local oceanographic conditions, and suggests population structuring on an ecological time scale.
- Overall, the study highlighted the discriminatory capacity of various methods and the need to use multiple approaches to determine the spatial ecology of highly migratory fish species.
- The contrasting results obtained here based on genetic and chemical approaches indicated that both provide information on different time scales: ecological for otolith chemistry and evolutionary for genetics.

## 6.2 Significance of the study

### 6.2.1 Mitogenome Characterization

The findings on the mitogenome enhance our understanding of its structure, detailing features of protein-coding regions, tRNA, rRNA, and non-coding regions, as well as comparative mitogenomics, phylogenetics, and evolutionary relationships within the Carangidae family. This information provides a valuable genetic resource and a foundational tool for further research in fisheries science.

- **Advanced understanding of trna and control regions in carangidae**: This study enhances our knowledge of the structural diversity in tRNA molecules and control regions within the Carangidae family, especially *D. russelli*. The detailed insights into tRNA stem and loop variations, as well as conserved motifs, expand our understanding of mitochondrial genome evolution in marine species.
- **Conservation of functional sites**: The discovery of highly conserved motifs like the mTERF binding site across evolutionary timescales highlights the significance of these regions in maintaining mitochondrial function. This conservation emphasizes the role of selective pressures in preserving critical mitochondrial functions across diverse species.
- **Evolutionary insights in carangidae phylogeny**: The study presents a refined phylogenetic analysis, revealing evolutionary relationships and divergence patterns within the Carangidae family, contributing valuable information on the evolutionary history of these fish.
- **Foundational data for future research**: By highlighting the necessity of including the subfamily Scomberoidinae in phylogenetic studies, the research sets a framework for

more comprehensive studies in marine genomics. This focus can help clarify unresolved phylogenetic questions and improve our understanding of genetic relationships within the family.

### 6.2.2 Population genetic studies

The present study conducted a comprehensive analysis of the population structure of the Indian Scad, *D. russelli* based on molecular markers and otolith chemical signatures offering valuable information for sustainable fisheries management of the species in the Indian Ocean region.

- **Understanding population connectivity:** This study provides essential insights into the genetic connectivity of *D. russelli* populations across the Indian Ocean. By demonstrating that populations are largely panmictic (genetically similar), it highlights the broad connectivity within this oceanic region, which can aid in understanding the dispersal patterns and resilience of the species.
- **Stock management for conservation:** The finding that *D. russelli* populations in the Indian Ocean can be managed as a single genetic stock has significant implications for fisheries management. Treating these populations as a single management unit simplifies management efforts and enables the sustainable harvesting of this species, ensuring its long-term viability.
- **Identification of genetic breaks and management units:** The observed genetic break between *D. russelli* populations in the Indian Ocean and the Indo-Malay Archipelago (IMA) suggests that these two regions should be managed separately. This differentiation allows for more targeted conservation and management strategies specific to each region's unique genetic background.
- **Historical insights into marine biodiversity:** The study provides valuable evidence that historical sea-level changes during the Pleistocene played a role in genetic divergence between Indian Ocean and IMA populations. This understanding enriches our knowledge of how past environmental shifts have shaped current genetic diversity in marine species.
- **Evidence of population expansion**: Genetic analyses, including Tajima's D, Fu's Fs, and mismatch analysis, indicate a recent population expansion or selective sweep. Understanding such demographic histories aids in anticipating population resilience and adaptability. These insights can be useful for predicting the species' resilience to future environmental changes and guiding conservation decisions accordingly.

- **Improving genetic tools in marine research:** The study's use of mitochondrial and nuclear markers, along with phylogenetic and haplotype network analyses, demonstrates the effectiveness of these methods in assessing genetic structure and connectivity. This approach can serve as a model for future research on other marine species, especially in large, interconnected oceanic regions.

### 6.2.3 Chemical analysis of otolith

This study also investigated the spatial structure and environmental differentiation of *D. russelli* populations along the Indian coast, utilizing otolith chemical analysis to assess ecological variation and comparing it to genetic data. By examining these factors on different time scales, the research offers a comprehensive view of population structuring in this migratory fish species.

- **Ecological differentiation**: Otolith chemical analysis revealed significant regional variation in elemental composition, highlighting ecological differentiation across *D. russelli* populations along the Indian coast. This reflects how local environmental conditions influence population structuring on an ecological time scale.
- **Insights into population structure**: The study demonstrates spatial structuring in *D. russelli* populations driven by environmental heterogeneity, suggesting that ecological forces like oceanographic conditions play a role in shaping population dynamics at regional scales.
- **Multi-method approach for clarity:** The findings emphasize the importance of using both genetic and chemical analyses to assess population connectivity, as each method provides distinct insights—genetics offering an evolutionary perspective and otolith chemistry capturing ecological interactions. By analyzing genetic connectivity and environmental influences on populations, the study provides an integrated approach to discerning population patterns that can be crucial for managing migratory marine species like *D. russelli*.
- **Guidance for fisheries management:** Understanding population structure on ecological and evolutionary scales can inform fisheries management policies that reflect regional environmental factors, ensuring sustainable practices that account for the species' spatial ecology.
- **Foundation for future studies:** this study's innovative combination of methods provides a model for future research in spatial ecology, contributing valuable

knowledge for the management and conservation of migratory fish species influenced by dynamic environmental conditions.

## 6.3 Societal Contributions of the study

- **Advancement in fisheries management:** By identifying that *D. russelli* populations in the Indian Ocean are genetically similar and can be managed as a single stock, this research simplifies management and conservation strategies. This contribution aids regulatory bodies in ensuring sustainable fishing practices that protect the long-term viability of *D. russelli*, a species significant to local and regional economies.
- **Informed conservation efforts:** The study's identification of a genetic break between Indian Ocean and Indo-Malay Archipelago populations allows targeted conservation strategies. Treating these regions as separate management units enables conservation policies to be tailored to the genetic characteristics and ecological needs of each population, promoting biodiversity and protecting ecosystem balance.
- **Enhanced understanding of environmental influences:** Through otolith chemical analysis, the study highlights how environmental factors, such as ocean currents and upwelling, shape population structures on an ecological scale. This knowledge can help local authorities consider environmental conditions in their management plans, promoting healthier marine ecosystems.
- **Insight into biodiversity and evolutionary history:** By analyzing the mitogenomic characteristics of *D. russelli* and its relatives, the study provides valuable evolutionary context for the Carangidae family. This understanding of biodiversity and genetic adaptation over time adds to our comprehension of how species evolve in response to changing environments, benefiting conservationists and biologists alike.
- **Foundation for future marine genomics research:** The study's findings on tRNA and control region structures, phylogenetic relationships, and genetic markers set a framework for further genomic studies. This contribution is especially valuable as it encourages continued research into the genetic foundations of marine species, helping to uncover the genetic basis of resilience and adaptability in marine ecosystems.
- **Public awareness of marine ecology**: This research underscores the complexity of marine species' connectivity and environmental differentiation. By revealing the intricate relationship between genetic and ecological factors, the study raises public

awareness of marine ecology and the importance of evidence-based management in maintaining ocean health.

- **Environmental policy**: The study underscores the ecological impact of environmental changes, such as monsoon-driven water currents, on marine species. This understanding can support policies that aim to preserve marine biodiversity by addressing factors influencing the coastal and oceanic environment, such as pollution control and habitat preservation
- **Impact on food security and livelihoods:** Effective fisheries management supported by this research can contribute to stable fish stocks, benefiting communities that rely on *D. russelli* for food and income. Sustainable management practices informed by this study help secure resources for future generations, supporting food security and economic stability.
- **Model for multi-method research in marine biology**: The study's use of genetic and otolith analyses on different time scales sets an example for future marine research. Such integrative approaches can be applied to other migratory species, helping scientists and policymakers better understand and manage marine populations.

**PUBLISHED / COMMUNICATED ARTICLES: 3**

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# Comparative mitogenomics and phylogenetics of the family Carangidae with special emphasis on the mitogenome of the Indian Scad *Decapterus russelli*

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**Carangids are abundant and commercially important marine fish that contribute to a significant portion of the fisheries in many parts of the world. In the present study, we characterized the complete mitogenome of the Indian scad, *Decapterus russelli* and performed a comprehensive comparative mitogenomic analysis of the family Carangidae. The comparative mitogenomics provided valuable insights into the structure, variability, and features of the coding and non-coding regions that evolved across species over millions of years. The structural features of tRNAs revealed changes in the frequency of mismatched and wobble base pairs, which is reflected in the base composition of H and L strands. The highly conserved sequence motif of the mTERF binding site in carangids over the ~ 400 MYA of their divergence demonstrated the functional importance of these sites. The control region of carangids was characterized by the presence of discontinuous repeat units with a high rate of sequence divergence in the form of base substitutions, insertions, and deletions. The maintenance of secondary structures in the control region independent of the rapid evolution of primary structure suggested the effect of selective constraints on their maintenance. Maximum likelihood (ML) and Bayesian inference (BI) phylogeny revealed a similar topology consistent with previous taxonomic studies. The extant carangids diverged through the evolutionary events experienced during the Cretaceous, Paleogene, and Neogene periods.**

The carangids represent an important group of exploited pelagic coastal resources of the world with diverse morphological features comprising approximately 147 species[1]. They occupy a variety of habitats consisting of pelagic, benthopelagic and reef-associated regions in all tropical and subtropical seas[2,3]. Currently, 4 subfamilies are recognized within the Carangidae[4]. Annual global capture production of Carangidae was estimated at 5.13 million tons in 2018, 65% of which came from Asia (https://www.fao.org/fishery/en/statistics). Among the carangids, one of the commercially most important species is the Indian scad, *D. russelli* (Ruppell, 1830), providing a cheap source of animal protein. It is also commonly used as live fish bait[5]. It is widespread in the Indo-Pacific and forms an important fishery resource in India and Southeast Asia[6]. Most studies on carangids have focused on biology, ecology, and taxonomy[7–18], and it is important to study the genetic and genomic traits to resolve taxonomic ambiguities, understand evolutionary traits, and implement conservation strategies.

The vertebrate mitochondrial genome, which plays a crucial role in cell metabolism and organism function, is ~ 15–20 kb in size and consists of 13 protein-coding genes (PCGs), 22 tRNA genes, 2 rRNA genes and a non-coding region (control region)[19]. The PCGs, tRNA and rRNA have a relatively low mutation rate because they are subject to strong purifying selection[20]. In contrast, the control region evolves at a high rate compared to the other parts of the mitogenome, and these high rates of sequence divergence are mostly localized in the variable flanking region, which is adjacent to the conserved sequence motifs[21–23].The extensive comparative studies in fish[24] have demonstrated the ability of the control region to form stable secondary structures and the existence

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of selective constraints for their maintenance. Although Zhu et al.[25] analyzed the phylogenetic relationships of carangids using the control region, there is a gap in knowledge regarding the mutational pattern and the non-random distribution of these mutations between the conserved motifs and their flanking regions. There is also a lack of knowledge about the structure and variability of tRNA genes in carangids and the frequency of occurrence of G-U wobble base pairs, the fundamental building block of RNA structure critical for RNA function in various biological systems.

The phylogenetic relatedness between the carangids has been extensively studied using comparative morphological[4,26–31] and molecular tools[32–37]. Although there has been a discourse on monophyly[4,28–31] vs. paraphyly[36,37] of carangids, the congruent topologies of phylogenetic trees constructed using DNA sequences suggested paraphyly of carangids if Echeneoids were not included[35–38]. Carangids can be considered as promising candidates for evolutionary studies, as they have a wide diversity in terms of habitat (marine, brackish water, freshwater), morphology (having variable body size; less than 20 cm to ~ 2 m), and shape (deep-bodied, streamlined). Some species of remoras live even as commensals on large pelagic vertebrates[39]. The phylogeographic study inferred from nuclear gene supermatrix suggested a terminal cretaceous crown age for carangids with major lineages diversifying during the Paleogene[36]. A late Eocene age has also been proposed for the crown carangids[35]. But densely sampled molecular studies indicated a Cenomanian stem age of the Carangoids which had undergone a significant diversification during the late cretaceous[37,38]. The subfamily Naucratinae originated early in the Paleocene[40], Trachinotinae date back to the early Oligocene and the remaining Carangoid lineages including the morphologically heterogeneous group, Caranginae comprising the genus *Decapterus* appear to have originated during the middle Eocene[38]. The genus *Decapterus* originated in the upper Miocene[41]. The genetic distance between the genus *Decapterus* was significantly greater than that between *Trachurus*[42], suggesting that *Decapterus* arose long before its sister group *Trachurus*, making them the most divergent species group. The morphology of the genus *Decapterus* includes multiple forms such as long-bodied, cylindrical, mackerel-like with a small mouth, reduced teeth and reduced scutes on the straight part of the lateral line, suggesting adaptations to the wide ranging off-shore life[43].

In the present study, we report the complete mitogenome of *D. russelli* for the first time elucidating the special characteristics. Although recent studies have examined the evolutionary history of carangids[44], we reanalyzed the patterns by including mitogenomes from 37 species and describing the relationships and evolutionary patterns among species with a robust phylogenetic tree. We also performed a comprehensive comparative analysis of carangid mitogenomes especially the mutational patterns of coding and non-coding regions, control region secondary structure, and structural features of tRNA to gain insights into the evolutionary dynamics of mitochondrial DNA.

## Results and discussion

**Mitochondrial genome organization and composition.** The total length of the mitochondrial genome of *D. russelli* was 16,542 bp. The genome organization is congruent with that observed in other teleost fishes with a standard set of 13 putative PCGs, 22 tRNA genes, 2 rRNA genes, and a control region. All genes were encoded on the heavy strand (H) except for the ND6 and 8 tRNA genes which were encoded on the light strand (L) (Supplementary Fig. S1). A similar gene arrangement was observed in other Carangidae species studied (Supplementary Table S1). The overall nucleotide composition of *D. russelli* mitochondrial genome was 27.5% A, 30.2% C, 25.4% T, and 16.9% G. The AT and GC skew values of the whole mitogenome were 0.039 and − 0.028 respectively.

The results suggested that A and C are predominant base constituents than T and G, with the overall nucleotide composition being severely C-skewed. These results agree well with their respective counterparts in other *Decapterus* spp.[45–47] The bias against G was presented following the results reported previously in other teleosts[48,49]. The AT-/GC- skew values of other Carangidae species were also determined (Supplementary Table S2). The sequence of the complete mitogenome of *D. russelli* has been deposited with NCBI, GenBank with the accession number MN711693.

**Overlapping and Intergenic spacer regions.** Seven gene overlapping regions ranging from 1–7 bp and 11 spacer regions varying in length from 1 to 38 bp were detected in the *D. russelli* mitogenome with the longest intergenic spacer region observed between tRNA Asn and tRNA Cys. Significant differences in the number and length of both the intergenic spacer region and the overlapping gene regions were observed in carangids (Supplementary Table S3). The number of spacer and overlapping gene regions varied between 10–12 and 6–15, respectively, between species. The intergenic spacer is distributed over 11 regions in almost all carangid species studied so far. However, an additional spacer region was observed in *C. armatus* (tRNA-His-tRNA-Ser (GCT)), *E. bipinnulata* (*ND6*-tRNA-Glu), *G. speciosus* (tRNA-Val-16SrRNA), *P. niger* (16SrRNA-tRNA-Leu (TAA)), *S. crumenophthalmus* (*ND6*-tRNA Glu) and *S. dumerili* (*ND6*-tRNA Glu). In addition, the spacer region found between tRNA-Arg and ND4L in all species was absent in *Seriola* spp, *S. nigrofasciata*, *T. carolinus* and *T. ovatus*. Besides, *C. equula* and *P. dentex* lacked a spacer region found between *ND1* and tRNA-Ile which was present in all other taxa. A comparative analysis among 37 carangids revealed that the longest spacer (42 bp) occurred between tRNA-Asn and tRNA-Cys of *T. carolinus*. Seven regions of overlapping gene regions were observed in most carangids, with the longest overlapping region being between ATP8 and ATP6 in some species and between ND4L and ND4 in others.

**Protein Coding Genes (PCGs).** The total length of PCGs in the *D. russelli* mitogenome was 11,425 bp, ranging in size from 165 bp (*ATP8*) to 1839 bp (*ND5*) accounting for 69% of the entire genome. GC skew values of all PCGs were negative except for the case with the L-strand encoded gene, ND6, which was greater than zero

| Locus | Strand | Position | Size (bp) | Codon | | A% | T% | G% | C% | A + T | G + C | AT skew | GC skew | Anticodon | Intergenic nucleotide |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Start | Stop | | | | | | | | | | |
| tRNAPhe | H | 1–68 | 68 | | | 35.3 | 19.1 | 23.5 | 22.1 | 54.4 | 45.6 | 0.297 | 0.030 | GAA | |
| 12SrRNA | H | 69–1022 | 954 | | | 30.8 | 21.4 | 22 | 25.8 | 52.2 | 47.8 | 0.180 | −0.079 | | |
| tRNA Val | H | 1023–1094 | 72 | | | 30.6 | 19.4 | 23.6 | 26.4 | 50 | 50 | 0.224 | −0.056 | TAC | |
| 16SrRNA | H | 1095–2817 | 1723 | | | 32.4 | 22 | 20.1 | 25.4 | 54.4 | 45.5 | 0.191 | −0.106 | | |
| tRNALeu (TAA) | H | 2818–2891 | 74 | | | 28.4 | 23 | 23 | 25.7 | 51.4 | 48.7 | 0.105 | −0.060 | TAA | |
| ND1 | *H* | 2892–3866 | 975 | ATG | TAA | 23.4 | 26.4 | 16 | 34.3 | 49.8 | 50.3 | −0.060 | −0.363 | | 5 |
| tRNA Ile | H | 3872–3941 | 70 | | | 27.1 | 21.4 | 28.6 | 22.9 | 48.5 | 51.5 | 0.117 | 0.110 | GAT | |
| tRNAGln | L | 3941–4011 | 71 | | | 35.2 | 25.4 | 15.5 | 23.9 | 60.6 | 39.9 | 0.161 | −0.210 | TTG | −1 |
| tRNA Met | H | 4011–4080 | 70 | | | 25.7 | 25.7 | 22.9 | 25.7 | 51.4 | 48.6 | 0.00 | −0.057 | CAT | |
| ND2 | H | 4081–5125 | 1045 | ATG | T– | 27.2 | 24.1 | 12.2 | 36.5 | 51.3 | 48.7 | 0.060 | −0.500 | | |
| tRNATrp | H | 5126–5196 | 71 | | | 32.4 | 16.9 | 25.4 | 25.4 | 49.3 | 50.8 | 0.315 | 0.00 | TCA | 1 |
| tRNAAla | L | 5198–5266 | 69 | | | 34.8 | 24.6 | 14.5 | 26.1 | 59.4 | 40.6 | 0.171 | −0.285 | TGC | 1 |
| tRNAAsn | L | 5268–5340 | 73 | | | 32.9 | 20.5 | 17.8 | 28.8 | 53.4 | 46.6 | 0.232 | −0.236 | GTT | 38 |
| tRNACys | L | 5379–5445 | 67 | | | 26.9 | 26.9 | 22.4 | 23.9 | 53.8 | 46.3 | 0.00 | −0.032 | GCA | |
| tRNA Tyr | L | 5446–5515 | 70 | | | 30 | 22.9 | 18.6 | 28.6 | 52.9 | 47.2 | 0.134 | −0.211 | GTA | 1 |
| COI | H | 5517–7067 | 1551 | GTG | TAA | 24.5 | 29.7 | 18.6 | 27.2 | 54..2 | 45.8 | −0.095 | −0.187 | | |
| tRNASer (TGA) | L | 7068–7138 | 71 | | | 28.2 | 23.9 | 19.7 | 28.2 | 52.1 | 47.9 | 0.082 | −0.180 | TGA | 3 |
| tRNA Asp | H | 7142–7212 | 71 | | | 29.6 | 28.2 | 21.1 | 21.2 | 53.8 | 46.3 | 0.026 | −0.002 | GTC | 7 |
| COII | H | 7220–7910 | 691 | ATG | T– | 27.9 | 25.3 | 16.6 | 30.1 | 53.2 | 46.7 | 0.049 | −0.289 | | |
| tRNA Lys | H | 7911–7984 | 74 | | | 31.1 | 21.6 | 20.3 | 27 | 52.7 | 47.3 | −3.614 | −0.141 | TTT | 1 |
| ATP8 | H | 7986–8150 | 165 | ATG | TAG | 24.1 | 26.8 | 13.9 | 35.2 | 50.9 | 49.1 | −0.053 | −0.430 | | −7 |
| ATP6 | H | 8144–8827 | 684 | ATG | TAA | 24.1 | 26.8 | 13.9 | 35.2 | 50.9 | 49.1 | −0.053 | −0.430 | | −1 |
| COIII | H | 8827–9611 | 785 | ATG | TA- | 23.9 | 26.6 | 17.8 | 31.6 | 50.5 | 49.4 | −0.053 | −0.280 | | |
| tRNAGly | H | 9612–9681 | 70 | | | 37.1 | 28.6 | 15.7 | 18.6 | 65.7 | 34.3 | 0.129 | −0.084 | TCC | |
| ND3 | H | 9682–10,030 | 349 | ATG | T– | 22.1 | 29.2 | 15.5 | 33.2 | 51.3 | 48.7 | −0.138 | −0.363 | | |
| tRNAArg | H | 10,031–10,099 | 69 | | | 31.9 | 33.3 | 18.8 | 15.9 | 65.2 | 34.7 | −0.021 | 0.083 | TCG | 1 |
| ND4L | H | 10,101–10,397 | 297 | ATG | TAA | 22.2 | 27.9 | 15.5 | 34.3 | 50.1 | 49.8 | −0.113 | −0.380 | | |
| ND4 | H | 10,391–11,771 | 1381 | ATG | T– | 25.6 | 25.8 | 14.7 | 34 | 51.4 | 48.7 | −0.003 | −0.396 | | |
| tRNA His | H | 11,772–11,843 | 72 | | | 29.2 | 29.2 | 19.4 | 22.2 | 58.4 | 41.6 | 0.00 | −0.067 | GTG | |
| tRNASer(GCT) | H | 11,844–11,911 | 68 | | | 25 | 17.6 | 25 | 32.4 | 42.6 | 57.4 | 0.173 | −0.128 | GCT | |
| tRNALeu(TAG) | H | 11,917–11,989 | 73 | | | 31.5 | 23.3 | 20.5 | 24.7 | 54.8 | 45.2 | 0.149 | −0.0930 | TAG | |
| ND5 | H | 11,990–13,828 | 1839 | ATG | TAG | 26.6 | 26.4 | 14.2 | 32.7 | 53 | 46.9 | 0.003 | −0.394 | | −4 |
| ND6 | L | 13,825–14,346 | 522 | ATG | TAG | 13.8 | 36 | 33.3 | 16.9 | 49.8 | 50.2 | −0.445 | 0.327 | | |
| tRNAGlu | L | 14,347–14,415 | 69 | | | 33.3 | 21.7 | 17.4 | 27.5 | 55 | 44.9 | 0.210 | −0.300 | TCC | 4 |
| Cytb | H | 14,420–15,560 | 1141 | ATG | T– | 23.7 | 27.1 | 15.8 | 33.5 | 50.8 | 49.3 | −0.070 | −0.352 | | |
| tRNAThr | H | 15,561–15,632 | 72 | | | 22.2 | 23.6 | 26.4 | 27.8 | 45.8 | 54.2 | −0.030 | −0.025 | TGT | −1 |
| tRNA Pro | L | 15,632–15,702 | 71 | | | 33.8 | 25.4 | 12.7 | 28.2 | 59.2 | 40.9 | 0.134 | −0.378 | TGG | |
| Dloop | H | 15,703–16,542 | 840 | | | 33 | 30.6 | 16.3 | 20.1 | 63.6 | 36.4 | 0.037 | −0.104 | | |

**Table 1.** Sequence characteristics of *Decapterus russelli* mitochondrial genome.

(Table 1). Similarly, the majority of PCGs had negative AT skew values. These results suggested that the bases C and T are over-represented in most PCGs. The base skew values for PCGs of the other 36 carangid species are presented in Supplementary Table S2. The GC and AT skew values of all were negative, except the AT skew value for *M. cordyla* and *C. tille* which was positive. Besides, *T. ovatus* was presented with equal numbers of A and T in PCGs, a trend not observed in other carangids.

The initiation and termination codons in *D. russelli* were confirmed by aligning the corresponding genes to other carangids. Among the 13 PCGs, 12 of them shared the same orthodox initiation codon, ATG, whereas *COI* had a different initiation codon and started with GTG instead of ATG (Table 1). Besides, 6 genes exhibited incomplete termination codons, either TA (*COIII*) or T (*ND2*, *COII*, *ND3*, *ND4*, and *Cyt-b*). The remaining genes revealed complete stop codons in open reading frames either TAA (*ND1*, *COI*, *ATP6*, and *ND4L*) or TAG (*ATP8*, *ND5*, and *ND6*). The incomplete codons can be completed by polyadenylation of the messenger RNA after cleavage[50,51]. Comparative analysis among carangids revealed that most PCGs started with the ATN initiation codon (Supplementary Table S4). *COI* of all the species started with GTG except ATC in *G. speciosus*. All other PCGs start with ATG except ATA for *ATP6* (*A. djedaba*, *A. kleinii*, *A. mate*, *C. ignobilis*, *C. melampygus*, *C. equula*, *C. tille and M. cordyla)* and *ND4L* (*T. trachurus*), and GTG for *ND5* (*T. blochii*, *T. carolinus* and *T. ovatus*).

We observed that the value of Ka was significantly smaller than Ks in all 13 PCGs on analyzing the evolutionary rate of PCGs. This indicates that there is a much lesser chance for a mutation to be different between species that can modify the amino acid sequences of a protein sufficiently to change its function than one which is silent[52]. The average Ka/Ks ratios substantially differed among PCGs indicating varying functional constraints experienced by individual genes (Supplementary Fig. S2; Supplementary Table S5). The fundamental features of genome evolution were largely determined by the influence of two non-adaptive forces, mutation pressure and genetic drift[53]. Nevertheless, the deleterious effect of mutation is very difficult to establish under purifying selection[54]. In PCGs, the average Ka/Ks ratio varied from 0.008 in *COI* to 0.469 in *ATP8*. Altogether the results indicated that the functional genes of carangids have undergone strong purifying selection to eliminate deleterious mutations thus maintaining the protein structure.

We estimated the degree of mitochondrial gene conservation by assessing the total p-genetic distance between 37 Carangidae species (Supplementary Fig. S3). In our analysis, we obtained the highest overall p genetic distance for the *ATP8* gene (0.301) and the lowest value for the *COI* gene (0.019) based on the data of the first and second nucleotide position of codons. The same trend was also observed in the values discerned from the full-length comparison of each gene, with *ATP8* and *COI* represented by a total p-genetic distance value of 0.390 and 0.134, respectively. Based on these results it could be inferred that gene, *ATP8* has the fastest evolutionary rate among Carangidae and *COI* the slowest. Besides, all genes had overall high p-genetic distance values for wobble nucleotide position, ranging from 0.393 to 0.564. This result was consistent with previous reports in other fish species that most of the differences occurred at the 3$^{rd}$ codon position of PCGs in mtDNA[55].

**Codon usage analysis.** A total of 3800 amino acids of PCGs are encoded in the mitogenome of *D. russelli*. The codon usage patterns are shown in Supplementary Fig. S4A. The amino acid Leu was represented by six different codons and the rest of them by either four or two. The amino acids Leu (16.46), Ala (8.75), and Thr (7.96) were most abundant in the *D. russelli* mitogenome. Among these, the frequency of Leu (CUC, CUA), Ala (GCC), and Thr (ACC) was highest. The frequencies of stop (UAG), Cys (UGU), and Arg (CGG) in PCGs were lowest. A comparative analysis showed that the codons ending in A or C occurred most frequently in amino acids with sixfold and fourfold degenerate third positions in Carangidae. In a two-fold degenerate codon, C was used more frequently than T. Consistent with the overall bias against G[56], it was the least common wobble position nucleotide in all categories. However, analysis of relative synonymous codon usage (RSCU) showed that the codons UCC for Ser, CUC and CUA for Leu and GCC for Ala occurred most frequently, while UUG for Leu, AGU for Ser and GCG for Ala were rare in the mitogenome of Carangidae (Supplementary Fig. S4B). It was also inferred from the RSCU value that the synonymous codons NNA and NNC were in the majority than the codons NNT and NNG (Supplementary Table S6).

**Transfer RNAs and ribosomal RNAs.** The mitogenome of *D. russelli* contained 22 tRNA genes typical of the metazoan mitogenome. In total, the tRNA genes contributed 1555 bp to the entire genome, which ranged from 67 bp (tRNA-Cys) to 74 bp (tRNA-Lys (TAA)). The secondary structure of tRNA genes predicted by ARWEN[57] makes it clear that all tRNAs could be folded into a canonical cloverleaf structure, with the exception of tRNA-Ser (GCT), in which no recognizable dihydrouridine (DHU) stem was found (Supplementary Fig. S5). Comparative analysis with other carangids revealed that the total length of tRNAs varied from 1548 (*S. rivoliana* and *E. bipinnulata)* to 1564 (*T. blochii*). The overall A + T content was estimated at 54.2%. Base skew analysis of 22 concatenated tRNA genes (Supplementary Table S2 revealed a positive AT skew (0.125) and a negative GC skew (− 0.098). The comparisons showed that the AT skew varied from 0.105 (*S. nigrofasciata*) to 0.224 (*T. ovatus*) and the GC skew varied from -0.120 (*S. rivoliana*) to − 0.091 (*T. carolinus*). The anticodons of these 22 tRNA genes were identical to those reported in other carangids (Supplementary Table S7). Despite the general one-to-one codon-anticodon correspondence, tRNA genes for Ser (TGA and GCT) and Leu (TAA and TAG) have been determined by two types of anticodons. Furthermore, G-U mismatches were observed for 16 of the tRNAs in their respective secondary structures (Supplementary Fig. S5). Five of the tRNA genes appeared to have A-C mismatches (tRNA-Val (TAC), tRNA-Leu (TAA), tRNA-Ile (GAT), tRNA-Arg (TCG), tRNA-His (GTG) in their respective amino acid acceptor stems. Similarly, two A-A mismatches, each for tRNA-Phe (GAA) and tRNA-Ser (GCT) and two C–C mismatches each for tRNA-Leu (TAG) and tRNA-Thr (TGT) were also detected. The presence of unmatched base pairs in mitochondrial tRNAs appeared to be a general trend that can later be repaired by post-transcriptional editing mechanisms prevalent in vertebrate mtDNA[58].

Two rRNA genes, a small (12 s rRNA) and a large (16 s RNA) subunit were located in the H strand of *D. russelli*. The total length of two concatenated rRNA genes was estimated to be 2677 bp with an A + T content of 53.7% (Supplementary Table S2). The AT content of the 12S rRNA gene was 52.2% with a positive AT skew of 0.172 and a negative GC skew of − 0.079. Similarly, the AT content of the 16S rRNA gene was 54.4% with a positive AT skew (0.191) and a negative GC skew (− 0.116). Moreover, the presence of a higher proportion of A and C bases in rRNA genes indicates an AC-rich trend, consistent with previous reports from other teleosts[53,59,60]. Consistent with previously reported results from other mitochondrial genomes[61–63], these two rRNA genes were positioned between tRNA-Phe and tRNA- Leu (TAA) separated by tRNA-Val. Comparative analysis among carangids (Supplementary Fig. S6) indicated that the length of two concatenated rRNA genes varied from 2655 bp (*G. speciosus*) to 2683 bp (*A. kleinii*). The AT skew varied from 0.1615 to 0.2256 and the GC skew varied from − 0.124 to − 0.077 (Supplementary Table S2).

**Features of tRNAs.** *Secondary structure.* The comparative analysis of 37 carangids revealed that most of the tRNAs are structurally different in both the stem and the loop. However, 2 tRNA genes, tRNA-Trp, and tRNA-Leu (TAG) exhibited similarities in their secondary structure (Fig. 1). As can be seen from Table 2, length

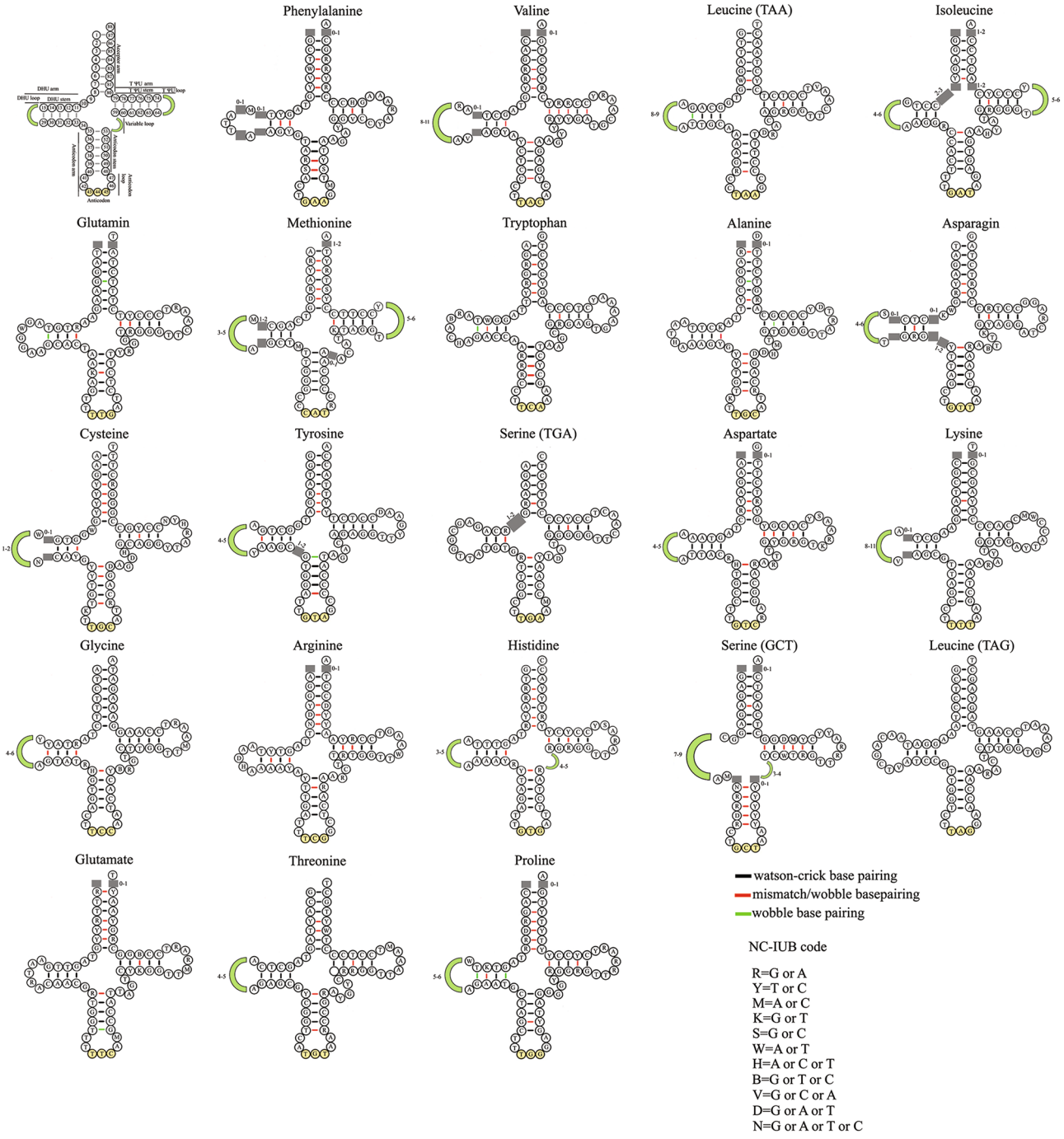


**Figure 1.** Secondary structure of tRNAs showing structural variations in 37 species. The first structure represents the nucleotide positions common to all tRNAs (1–88) and details of stem- loop. Length variations in stems and loops are indicated by grey squares and green arcs respectively. The secondary structure of tRNAs were predicted by ARWEN software (http://130.235.244.92/ARWEN/) and edited manually in Adobe Photoshop CS6.0.

variation was detected in all four stems. The anticodon stem (AC stem) of all the tRNAs was 5 bp, except for tRNA-Ser (GCT), which displayed length variation (5 or 6 bp). Though the pseudouridine (TΨU) stem of most tRNAs was 5 bp, tRNA-Phe (4 bp), tRNA-Met (4 or 5 bp), tRNA-Lys (4 bp), and tRNA-Ser (GCT) (6 bp) indicated length variation. In the acceptor stem (AA stem), tRNA-Ser (TGA) displayed a length of 6 bp, and the rest of the tRNAs showed more of a length variation of either 7 bp or a range of 7 to 8 bp. Although the DHU stem was mostly 4 bp in length with a range of 3 to 5 bp, tRNA-Ser (GCT) of all carangids examined had only a small loop with no stem.

Of the four loops, the length of the AC loop was fixed (4 nucleotides), whereas the remaining three loops showed considerable variation in length. Of these, the DHU loop was extremely variable, ranging from 3–13

| | AA stem (bp) | | | DHU stem (bp) | | | | DHU loop (nucleotide) | | | | | | | | | | | AC stem (bp) | | V loop (nucleotide) | | | TΨU stem (bp) | | | TΨU loop (nucleotide) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| tRNA | 6 | 7 | 8 | 0 | 3 | 4 | 5 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 5 | 6 | 3 | 4 | 5 | 4 | 5 | 6 | 6 | 7 | 8 | 9 |
| **H strand coded** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Phe | | 35 | 2 | | 12 | 25 | | | | 24 | | 13 | | | | | | | 37 | | | 37 | | 37 | | | | | 37 | |
| Val | | 35 | 2 | | 33 | 4 | | | | | | | 4 | | 33 | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Leu(TAA) | | 37 | | | | 37 | | | | | | | | | 21 | 16 | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Ile | | 18 | 19 | | 37 | | | | | | 1 | 36 | | | | | | | 37 | | | | 37 | | 37 | | | 26 | 11 | |
| Met | | 2 | 35 | | 8 | 29 | | | | 7 | 18 | 12 | | | | | | | 37 | | | 34 | 3 | 1 | 36 | | | 36 | 1 | |
| Trp | | 37 | | | | 37 | | | | | | 36 | 1 | | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Asp | | 36 | 1 | | | 37 | | | | | 3 | 34 | | | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Lys | | 33 | 4 | | 36 | 1 | | | | | | | | | 1 | 6 | 29 | 1 | 37 | | | | 37 | 37 | | | | | | 37 |
| Gly | | 37 | | | | 37 | | | | | 28 | 6 | 3 | | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Arg | | 31 | 6 | | | 37 | | | | 37 | | | | | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| His | | 37 | | | | 37 | | | | 3 | 2 | 32 | | | | | | | 37 | | | 11 | 26 | | 37 | | | 37 | | |
| Ser(GCT) | | 3 | 34 | 34 | | | | | | | | | | | | 6 | 30 | 1 | 7 | 30 | 31 | 6 | | | | 37 | | 37 | | |
| Leu(TAG) | | 37 | | | | 37 | | | | | | | 37 | | | | | | 37 | | | | 37 | | 37 | | | 37 | | |
| Thr | | 37 | | | | 37 | | | | | 3 | 32 | 2 | | | | | | 37 | | | | 37 | | 37 | | | 37 | | |
| **L strand coded** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Gln | | 32 | 5 | | | 37 | | | | | | 37 | | | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Ala | | 20 | 17 | | | 37 | | | | 37 | | | | | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Asn | | 37 | | | | 27 | 10 | | | | 14 | | 23 | | | | | | 37 | | | | 37 | | 37 | | | 37 | | |
| Cys | | | 37 | | 5 | 32 | | 23 | 14 | | | | | | | | | | 37 | | | 37 | | | 37 | | 2 | 35 | | |
| Tyr | | 37 | | | | 37 | | | | | 34 | 3 | | | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Ser(TGA) | 37 | | | | 37 | | | | | | | | | 37 | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Glu | | 32 | 5 | | | 37 | | | | 37 | | | | | | | | | 37 | | | 37 | | | 37 | | | 37 | | |
| Pro | | 32 | 5 | | | 37 | | | | | | 36 | 1 | | | | | | 37 | | | 34 | | | 37 | | | 37 | | |

**Table 2.** Size of stems and loops of 22 tRNA genes in the mitogenomes of 37 species of Carangidae.

nucleotides. Conversely, the variable loop (V loop), mostly 4 nucleotides with a range of 3–5, and T ΨU loop, mostly 7 nucleotides with a range of 6–9 displayed the least variation.

*Wobble base pairs in the stem regions.* The wobble base pair is a unique feature of RNA structure and their often high evolutionary conservation[64] underscores their functional and structural importance. Due to its thermodynamic stability, the G-U often substitutes G-C or A-U base pairs and it differs from Watson–Crick and other mismatched base pairs owing to its structural, conformational, and functional properties[65]. Diverse proteins and other ligands specifically targeting tRNA exploit these features[66] and thus the comparative analysis of tRNAs is very crucial for understanding the structural and functional features of the mitogenome. Supplementary Table S8 illustrates the frequencies of Watson–Crick and Wobble pairing at each of the 25 base pair sites of each tRNA in 37 carangids (8 in AA stem + 5 in DHU stem + 6 in AC stem + 6 in TΨU stem). Seven sites from each stem (1–87, 2–86 and 8–80 base pair sites in the AA stem, 12–32 and 15–30 in the DHU stem, 40–48 in the AC stem, and 64–74 in the TΨU stem) revealed a high rate of (100%) Watson–Crick and Wobble base pairing (hydrogen bonding) and these sites may play a pivotal role in maintaining stem structure. Among the tRNAs, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Ser (TGA), tRNA-Asp, and tRNA-Pro had a higher frequency (≥ 98% overall average) of hydrogen bonding than other tRNAs. Moreover, L strand-encoding tRNAs exhibited, on average, a higher frequency of hydrogen bonding (97.48%) than H strand encoding tRNAs (94.78%).

Table 3 shows the number of G-U wobble base pairs in each stem of 22 tRNAs for the 37 carangid species. Individual stems and tRNAs rather displayed considerable variations in the frequency of GU base pairs, 6% of the base pairs comprised of wobble base pairs on average in the four stems of mitochondrial tRNA with a large difference between stems (4.57% in the TΨU stem and 12.26% in the DHU stem). A substantial difference was also discerned between the tRNAs (0.72% in tRNA-Ile versus 19% in tRNA-Glu) with no wobble base pairs observed in the case of tRNA-Lys and tRNA-Leu (TAG). Furthermore, the tRNAs encoded by different strands exhibited a significant difference in the frequency of wobble base pairs, as the L- strand encoded tRNAs had a higher frequency of wobble base pairs (13.18%) than those of H- strand encoded tRNAs (3%) (Mann–Whitney U test, $p < 0.01$).

*Mitochondrial transcription termination factor (mTERF) binding site.* We compared tRNA-Leu (TAA) which has been reported to contain the target binding site for mTERF in humans[67] to identify its presence in Carangidae. An important tridecameric sequence (5'TGGCAGAGCCCGG3') reported in the human mitochondrial tRNA-Leu (TAA) gene[48,49] was also identified in Carangidae in the same region as the corresponding part of

| | tRNA | AA stem | DHU stem | AC stem | TΨU stem | Total | |
|---|---|---|---|---|---|---|---|
| | | 5871 | 3066 | 4066 | 3999 | | |
| | Total base pairs | Wobble bp | Wobble bp | Wobble bp | Wobble bp | 17,002 | Percentage |
| H strand coded | Phe | 10 | 6 | 0 | 1 | 17 | 2.5 |
| | Val | 2 | 2 | 0 | 3 | 7 | 0.93 |
| | Leu(TAA) | 0 | 37 | 0 | 3 | 40 | 4.6 |
| | Ile | 6 | 0 | 0 | 0 | 6 | 0.72 |
| | Met | 33 | 0 | 0 | 11 | 44 | 5.50 |
| | Trp | 2 | 35 | 0 | 0 | 37 | 4.80 |
| | Asp | *2* | 0 | 0 | 17 | 19 | 2.50 |
| | Lys | 0 | 0 | 0 | 0 | 0 | 0 |
| | Gly | 0 | 65 | 0 | 0 | 65 | 8.40 |
| | Arg | 8 | 6 | 2 | 18 | 34 | 4.34 |
| | His | 2 | 7 | 0 | 23 | 32 | 4.20 |
| | Ser(GCT) | 0 | | 5 | 7 | 12 | 1.64 |
| | Leu(TAG) | 0 | 0 | 0 | 0 | 0 | 0 |
| | Thr | 0 | 0 | 6 | 0 | 6 | 0.80 |
| | Average (%) | 1.72 | 8.27 | 0.50 | 3.29 | 3.00 | |
| L strand coded | Gln | 37 | 37 | 0 | 0 | 74 | 9.46 |
| | Ala | 42 | 30 | 41 | 34 | 150 | 18.82 |
| | Asn | 24 | 37 | 0 | 18 | 79 | 10.03 |
| | Cys | 52 | 34 | 1 | 1 | 88 | 10.81 |
| | Tyr | 37 | 0 | 37 | 0 | 74 | 9.52 |
| | Ser(TGA) | 3 | 6 | 29 | 34 | 66 | 9.40 |
| | Glu | 68 | 0 | 71 | 9 | 148 | 19.00 |
| | Pro | 35 | 74 | 32 | 1 | 142 | 18.13 |
| | Average | 14.13 | 18.84 | 14.25 | 6.75 | 13.18 | |
| | Coefficient of varitaion | 1.30 | 1.29 | 1.84 | 1.32 | 0.88 | |
| | Grand average | 6.18 | 12.26 | 5.50 | 4.57 | 6.70 | |

**Table 3.** Occurrence of wobble pairings in the stem regions of 22 tRNA genes in the mitogenome of 37 species of Carangidae.

their DHU arm. The mTERF binds to this site with high affinity and regulates the level of transcription from the rRNA genes into the remaining genes of the major coding strand[50].

All bases in the 13 nucleotide positions identified in 37 carangid taxa were completely identical to those in humans (Supplementary Fig. S7), suggesting that this site in carangids may follow the same mechanism of function as proposed for humans.

*Control region: mutational patterns and secondary structure.* The control region of *D. russelli* was shorter than other carangids, consisting of 840 bp flanked by tRNA-Pro and tRNA-Phe on either side. The control region comprised the highest A + T content (63.6%) compared to other regions of the mitogenome (Supplementary Table S2). AT skew was positive (0.038) and GC skew was negative (− 0.104). Considerable variation was observed in the AT and GC skew values of other carangids, ranging from − 0.047 (*S. crumenophthalmus*) to 0.065 (*T. blochii*) and − 0.278 (*C. ignobilis*) to − 0.093 (*D. maruadsi*), respectively. The composition of the nucleotide adenine (A) is higher than that of thymine (T) in *D. russelli* compared to other carangids (Supplementary Table S2). However, the control region of *A. mate*, *A. djedaba, C. ignobilis, G. speciosus,* and *T. blochii* possess a slightly higher percentage of adenine (A) than that of *D. russelli*. Besides, the composition of thymine (T) is higher than adenine (A) in *C. armatus, C. malabaricus, C. tille, C. melampygus, T. japonicas, S. crumenophthalmus, S. dumerili,* and *S. lalandi*. The GC skew of all the carangids was negative. It was found that the maximum length variation for the control region was observed in the range of 840 bp (*D. russelli*) to 904 bp (*S. crumenophthalmus*) in the carangids examined. Among the carangids, *S. crumenophthalmus* possessed the longest mitogenome, and *S. rivoliana* and *S. dumerili* were determined to have the shortest mitogenome. It was evident that these species do not exhibit any notable variation with respect to their PCGs, tRNA and rRNA (Supplementary Fig. S6), hence the 61–62 bp variation in the control region alone contributed to the difference in their overall mitogenome length. The majority of the primary sequences of the control region are presumed to be not involved in regulatory function as this region shows great variation even among closely related species[68].

Comparative mitogenomics of carangids revealed high variability in the mitochondrial control region due to length variations and the accumulation of base substitutions and indels. Consistent with the recognition sites in many marine fish[69] we have detected the 3 central conserved sequence blocks (CSB- F, CSB- E, and CSB-D) and 3 conserved sequence blocks (CSB- 1, CSB-2, and CSB- 3) in all taxa. Palindromic sequence motifs—'TACAT'

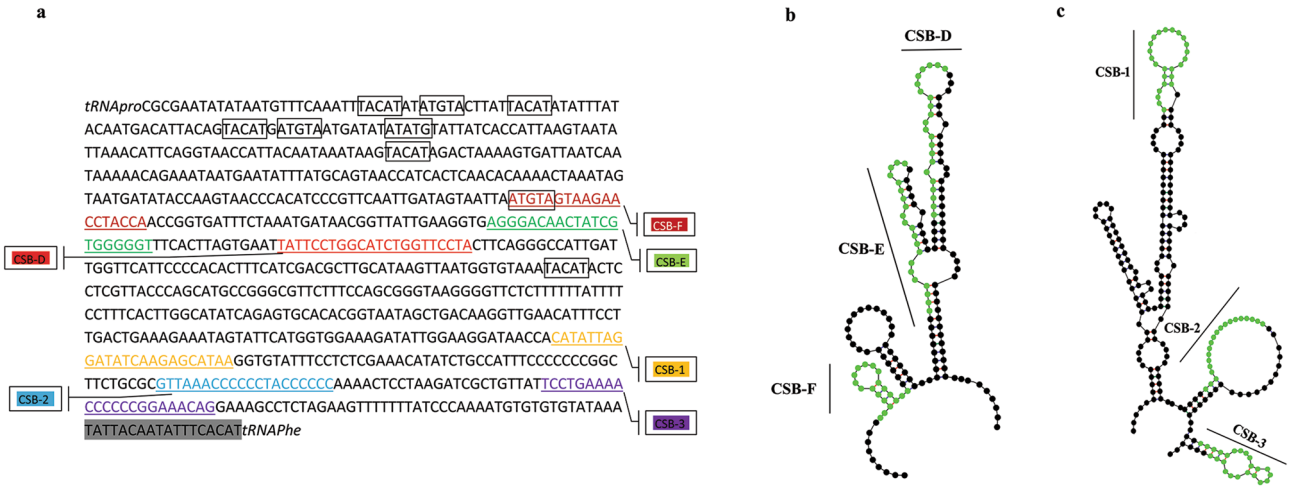


**Figure 2.** Sequence and structure of the mitochondrial DNA control region of carangids (**a**) Features of the control region. CSB-F,-D,-E: central conserved sequence blocks; CSB-1,-2,-3: conserved sequence blocks. Palindromic sequence motifs 'TACTA' and 'ATGTA' are framed. Grey colored bar represents the characteristic 3' terminal sequence. (**b**) The secondary structure identified in CSB-F, CSB-D, and CSB -E. (**c**) Secondary structure identified in CSB-1, CSB-2, and CSB-3. The secondary structures of conserved sequence motifs were predicted by the online Mfold web server (http://www.unafold.org/).

and 'ATGTA' were found in multiple copies distributed towards the 5' end of the control region [Fig. 2; Supplementary Table S9] and are believed to act as the termination site of heavy strand replication[69]. The segment corresponding to Extended Termination Associated Sequence (ETAS) previously reported in *Scomberoides commersonianus*, a member of the Carangidae[25], could not be aligned to the corresponding segment in the control region of either species.

No tandem repeat sequences were detected in the control region of Carangidae, but the T homopolymer of 5–12 nucleotides between CSB-D and CSB-1 was found in all taxa. Along with these discontinuous multiple interval repeats of ATATTA and TATAAT were also observed in the control region of Carangidae.

All species except *D. maruadsi* displayed substantial differences from the key sequences of CSB- E[25] and all were substitutions ranging from 1 to 9 nucleotides. Furthermore, *M. cordyla, P. dentex,* and *Caranx* spp. lacked a 'GTGGG'- box which is a typical feature of CSB- E in teleosts[69]. The CSB- D was highly conserved among carangids and exhibited high sequence similarity between species (Table 4). Although the functions of the CSBs remain unclear, CSB-D is highly conserved in fish families and is involved in regulation of H-strand replication and initiation of D-loop structure[70] and could play a potential role in mitochondrial metabolism[66]. Conserved sequence blocks (CSB-1,-2, and -3) exhibit a higher level of divergence in carangids with interspecies substitutions, insertions, and deletions (Table 4). Except for *P. dentex*, S. *nigrofasciata, S. leptolepis* and *Seriola* spp. other species showed a characteristic sequence at the 3' end (Table 4). These variations imply rapid evolution of the primary structure in the Carangidae control region, which can provide information for understanding their structural and functional relationship. Furthermore, these conserved regions are involved in the formation of secondary structures in the control region (Fig. 2) that serve as the target sites for proteins or enzymes[69]. These conserved sequence motifs formed similar secondary structures in Carangidae although their primary sequences are not conserved. Therefore, the data reinforce that although there is sequence divergence in the conserved sequence motif, their structural elements and locations appeared to be conserved in Carangidae and some selective constraints on these regions acted to maintain their structure and function.

*Carangidae mitogenome sequence comparison.* We compared the complete mitochondrial genome of *D. russelli* with other available mitogenomes of the Carangidae family. The DNA coding sequences showed high interspecies identity (Supplementary Figs. S8, S9). The control region contributed to a larger percentage difference between the nucleotide sequences. It has been found that *ND1*, *COI*, *COII* and *COIII* are highly conserved among the DNA coding sequences. In addition, ATP8 was the least conservative of all species, in good agreement with the results obtained from analysis of Ka/Ks and overall mean p genetic distance. With the exception of ND6 and Cyt-b, other PCGs expressed orthologous group (COG) clusters. A total of 12 COGs of proteins were detected from11 PCGs. *ND5* was determined to have 2 COGs, pCOG, and cCOG, and the remaining 10 PCGs each expressed a single COG each (cCOG). COGs are a group of proteins inferred to be orthologous i.e. they are direct evolutionary counterparts[71]. Lineage-specific duplications (orthologous relationships) exist mainly between gene (protein) families rather than between individual proteins[72]. The identification of COGs associated with specific PCGs could indicate the presence of a specific protein in a specific species, and it is crucial to determine the functional relevance of these proteins in that specific species[73]. The results indicated that the COGs present in carangids are involved in inorganic ion transport and metabolism (pCOG) and energy production and conservation (cCOG)[72]. The ND5 gene has been found to perform these two functions, while energy metabolism is the sole function of the remaining 10 PCGs.

| Species | Central conserved blocks | | |
|---|---|---|---|
| | CSB- F | CSB- E | CSB- D |
| Reference sequence | ATGTAGTAAGAACCTACCA | AGGGACAACTATTGTGGGGGT | TATTCCTGGCATTTGGTTCCTA |
| *Dr* | ********** | **********C******* | **********C******** |
| *Ac* | **********G******* | *******GAA********** | ********** |
| *Ai* | ********** | **********G | ********** |
| *Ad* | ********** | ********TA********** | ********** |
| *Ak* | ********** | ********AA********** | ********** |
| *Am* | ********** | *******GAA********** | ********** |
| *Ca* | ********** | ********AA********** | ********** |
| *Cb* | ********** | *******GAA********** | ********** |
| *Ce* | ********** | ********AA*CC******** | ********** |
| *Cm* | ********** | *A******AA******A**** | ********** |
| *Cp* | GCCC*A********** | *******GAA********** | ********** |
| *Ci* | **A****G*AGG*TC**** | ***A*****C***ACATTC*C | ********** |
| *Ct* | *C******G**C*****GG | *A******AA**C***A**** | **********C***** |
| *Cme* | *C******G**C*****GG | *A******AA******A**** | **********C***** |
| *Dmac* | ********** | ********AA********** | **********C******** |
| *Dm* | **********G***** | ********AA********** | ********** |
| *Dmar* | ********** | ********** | **********C******** |
| *Dt* | ********** | ********AA**C******** | ********** |
| *Eb* | ***C********** | *******GTA********** | ********** |
| *Gs* | **********G******* | *******GAA********** | **********C***** |
| *Mc* | *C*C*********C**** | *A*****GAA******A**** | **********C***** |
| *Pn* | ********** | ********AA********** | ********** |
| *Pd* | ********** | ********AA*****A***** | ********** |
| *Tt* | **********G******* | ********AA**C******** | ********** |
| *Tj* | **********G******* | ********AA**C******** | ********** |
| *Sc* | ********** | G*A****GAA**C******** | ********** |
| *Sd* | *CTC*A********G**** | *******GAA**C******** | ********** |
| *Sl* | *CTC*A********G**** | *******GTA********** | ********** |
| *Sq* | *CCC*A********G**** | ********TA**C******** | ********** |
| *Sle* | ********** | *A*****GAA********** | ********** |
| *Sr* | ********** | *******GAA********** | ********** |
| *Sn* | *CCC*A********G**** | ********TA**C******** | ********** |
| *Tb* | **********G******* | ********AA**C******** | ********** |
| *To* | GCAC*******GA*C**** | ********A*C********* | ********** |
| *Tc* | **********G******* | ********TA********** | ********** |
| *Uh* | ********** | *******GAA********** | ********** |
| *Us* | ********** | ******AGAA********** | ********** |
| **Species** | **Conserved sequence blocks** | | |
| | **CSB- 1** | **CSB- 2** | **CSB- 3** |
| Reference sequence | CATATTAGGATATCAAGAGCA TAA | GTAAAACCCCCCTACCCCCC | TCCTGAAAACCCCCCGGA AAAAA |
| *Dr* | ********** | **T********** | **********C*G |
| *Ac* | *****A*A*********TA***** | **T********** | **********C*G |
| *Ai* | **********T****** | **T********** | **T**********C*G |
| *Ad* | ****C*TT********** | ********** | **T**********C*G |
| *Ak* | ******T********** | **********T | **********C*G |
| *Am* | ******T********** | ********** | **********C*G |
| *Ca* | **********TA***** | ********** | **********C*G |
| *Cb* | **********T****** | ********** | A**********C*G |
| *Ce* | *****A**********GA***** | *****A******C******T | **********A****C*G |
| *Cm* | **********T****** | ********** | **********C*G |
| *Cp* | **********T****** | ********** | **********C*G |
| *Ci* | *******TT***C********** | ********AC******C******* | **********C*G |
| *Ct* | *******TTT********** | ********** | **********C*G |
| *Cme* | *******TTT********** | *****A********** | **********C*G |
| Continued | | | |

| Species | Conserved sequence blocks | | |
|---|---|---|---|
| | CSB- 1 | CSB- 2 | CSB- 3 |
| *Dmac* | ********** | **********T | **********C*G |
| *Dm* | ********** | **********T | **********C*G |
| *Dmar* | ********** | ********** | **********C*G |
| *Dt* | ********** | **********T | **********C*G |
| *Eb* | *****CT********** | **T********** | **********C*G |
| *Gs* | **C****AT*********T****** | ********** | **********C*G |
| *Mc* | *******TA********** | **T********** | **********C*G |
| *Pn* | *****ATT*********T****** | **T*********C******T | **********C*G |
| *Pd* | ***C***A*********GA***** | C*C**********C****** | *T**A**G*T****GAA*GG*C* |
| *Tt* | ********** | **********T | **********C*G |
| *Tj* | ********** | **********T | **********C*G |
| *Sc* | ****CGT********** | ***********CCA***** | **********C*G |
| *Sd* | ******G********** | T********** | **********G***C* |
| *Sl* | ********** | ******A********** | *********A****T*******C* |
| *Sq* | *******A*******T******** | ******A********** | *********A****TT******C* |
| *Sle* | **C****CT********** | ********** | **********C*G |
| *Sr* | *****A********** | T********** | **********G***C* |
| *Sn* | *****AGA*******T******** | ********** | **********T***C*G |
| *Tb* | ****A*T*********T****** | ***G********** | **********C*G |
| *To* | ****A*TA*********T****** | ***********C******* | **********C*G |
| *Tc* | G*C****ATA*********T****** | ******A********** | **********C*G |
| *Uh* | ********** | **********T | **********C*G |
| *Us* | ********** | **********T | **********C*G |
| **Species** | **3' end terminal sequence** | | |
| Reference sequence | TATTATAATATTTCACAT | | |
| *Dr* | *****C********* | | |
| *Ac* | ********** | | |
| *Ai* | ********** | | |
| *Ad* | ********** | | |
| *Ak* | ********** | | |
| *Am* | ********** | | |
| *Ca* | ********** | | |
| *Cb* | ********** | | |
| *Ce* | ********** | | |
| *Cp* | ********** | | |
| *Cm* | ********** | | |
| *Dmac* | *****C********* | | |
| *Dm* | *****C********* | | |
| *Dmar* | *****C********* | | |
| *Dt* | *****C********* | | |
| *Eb* | *****A********* | | |
| *Gs* | ********** | | |
| *Mc* | ********** | | |
| *Pn* | ********** | | |
| *Pd* | absent | | |
| *Ci* | ********** | | |
| *Ct* | ********** | | |
| *Cme* | ********** | | |
| *Tt* | ********** | | |
| *Tj* | ********** | | |
| *Sc* | *****C********T** | | |
| *Sd* | Absent | | |
| *Sl* | Absent | | |
| *Sq* | Absent | | |
| *Sle* | Absent | | |
| Continued | | | |

| Species | 3' end terminal sequence | | |
|---|---|---|---|
| *Sr* | Absent | | |
| *Sn* | Absent | | |
| *Tb* | **********G**T** | | |
| *To* | **********T** | | |
| *Tc* | **********G**T** | | |
| *Uh* | ********** | | |
| *Us* | ********** | | |

**Table 4.** The comparison of Central conserved blocks, Conserved sequence blocks and 3' terminal region in 37 species of Carangidae. *Dr* = *D. russelli, Ac* = *A. ciliaris, Ai* = *A. indica, Ad* = *A. djedaba, Ak* = *A. kleinii, Am* = *A. mate, Ca* = *C. armatus, Cb* = *C. bajad, Ce* = *C. equula, Cm* = *C. malabaricus, Cp* = *C. plagiotaenia, Ci* = *C. ignobilis, Ct* = *C. tille, Cmel* = *C. melampygus, Dmac* = *D. macerellus, Dm* = *D. macrosoma, Dmar* = *D. maruadsi, Dt* = *D. tabl, Eb* = *E. bipinnulata, Gs* = *G. speciosus, Mc* = *M. cordyla, Pn* = *P. niger, Pd* = *P. dentex, Tt* = *T. trachurus, Tj* = *T. japonicas, Sc* = *S. crumenophthalmus, Sd* = *S. dumerili, Sl* = *S. lalandi, Sq* = *S. quinqueradiata, Sle* = *S. leptolepis, Sr* = *S. rivoliana, Sn* = *S. nigrofasciata, Tb* = *T. blochii, To* = *T. ovatus, Tc* = *T. carolinus, Uh* = *U. helvola, Us* = *U. secunda.* Asterisks indicate the same nucleotide as the reference sequence. Insertions are represented as bold and deletions as underlined. Other nucleotides denote base substitutions.

*Phylogenetic analysis.* The phylogenetic position of *D. russelli* is shown in Fig. 3 relative to 36 species of carangids based on 1380 bp sequences. Analogous topologies were obtained for both maximum likelihood analysis (ML) and Bayesian inference (BI) with almost 100% bootstrap support and strong posterior probability values, respectively (Fig. 3). The result implied that 37 species formed 3 lineages corresponding to the subfamilies Caranginae, Naucratinae, and Trachinotinae. The reconstructed phylogenetic tree places *D. russelli* in the same branch with other *Decapterus* spp. and forms a sister group to the genus *Trachurus* and the findings support the previous classification of carangids[44,74]. The phylogenetic tree recovered the three carangid subfamilies as monophyletic and it also supported the groupings (Trachinotinae + (Naucratinae + Caranginae)). This result confirmed the phylogenetic relationship of carangids described by previous findings based on morphological synapomorphic characters[4,31] and partial sequences of mitochondrial genes[32,75]. Within the Naucratinae, the monotypic *E. bipinnulata* branched individually early from *Seriolina* and *Seriola* spp. After diverging from *Seriolina,* all *Seriola* species formed a monophyletic clade. Furthermore, both phylogenetic trees indicated two well-separated lineages in Caranginae. The first lineage included the genus *Selar, Trachurus,* and *Decapterus.* This lineage also encompassed the species *C. equula* on the same branch with *P. dentex* in contrast to other *Carangoides* Spp. which were grouped into a different branch (Fig. 3). This result confirms the findings of previous studies[44,76] and thus provides compelling evidence for the assignment of *C. equula* to its previously described genus, *Kaiwarinus*. The second lineage was the most taxonomically diverse group of the family Carangidae delineated in the present study as it consisted of 10 genera divided into two larger groups. The first lineage comprised the genera: *Caranx* + *Megalaspis* + *Gnathandon* as well as *Atule* + *Alepes.* The latter also included the monotypic *S. leptolepis* which individually branched off from this lineage early. The second lineage included *Uraspis* + *Parastromateus* and *Alectis* + *Carangoides.* In addition, a paraphyletic relationship between *Caranx* + *M. cordyla, Alepes* + *A. mate,* and *Uraspis* + *P. niger* was also observed.

*Divergence time estimation.* The RelTime ML analysis revealed that the differentiation of *D. russelli* could be dated back to about 4.69 Mya in the early Pliocene. The family Carangidae had diverged from other lineages when the Cretaceous–Paleogene extinction event occurred and it was estimated to be around 79 Mya towards the end of the cretaceous (Supplementary Fig. S10). It was observed that the separation of the two groups, Trachinotinae and (Caranginae + Naucratinae) occurred at 74 Mya at the end of the Cretaceous. Further, the split between the subfamilies Caranginae and Naucratinae occurred at 62.20 Mya (Early Paleocene). Although the diversification of most species after the Cretaceous mass extinction occurred in the Paleogene[75], several more recent Neogene radiations can also be observed (Fig. 4).The most recent split was observed at 0.34 Mya between the two *Uraspis* species. Our analyzes of the diversification of the genus Trachurus (18.40 Mya, early Miocene) were consistent with previous studies based on the data of morphological characters and ecological aspects[77] and cyt b gene sequence analysis[78]. The split between *Seriola* + *Seriolina* and *Elgatis* occurred during the early Paleocene, consistent with Swart et al.[40] but much younger than Le et al.[44]. The radiation of *Seriola* appears to be 20.87 Mya which is in direct agreement with Le et al.[44], but slightly predates the estimates by Santini and Carnevale[38].

## Conclusion

The present investigation focused on the characterization of the complete mitochondrial genome of the Indian Scad, *D. russelli*, and a comprehensive comparative analysis of Carangidae with special emphasis on the structure and variability of tRNA and control region. The carangid tRNA was structurally different in both the stem and loop, with the exception of tRNA-Trp, and tRNA-Leu (TAG). These structural features resulted in mismatched or wobble base pairs in tRNAs. The high rate of Watson–Crick and Wobble base pairing in the stem regions of tRNA suggested their role in stem structure maintenance. The classical cloverleaf secondary structure was retained in all tRNAs except tRNA-Ser (GCT). The presence of an mTERF binding site and a very high evolutionary

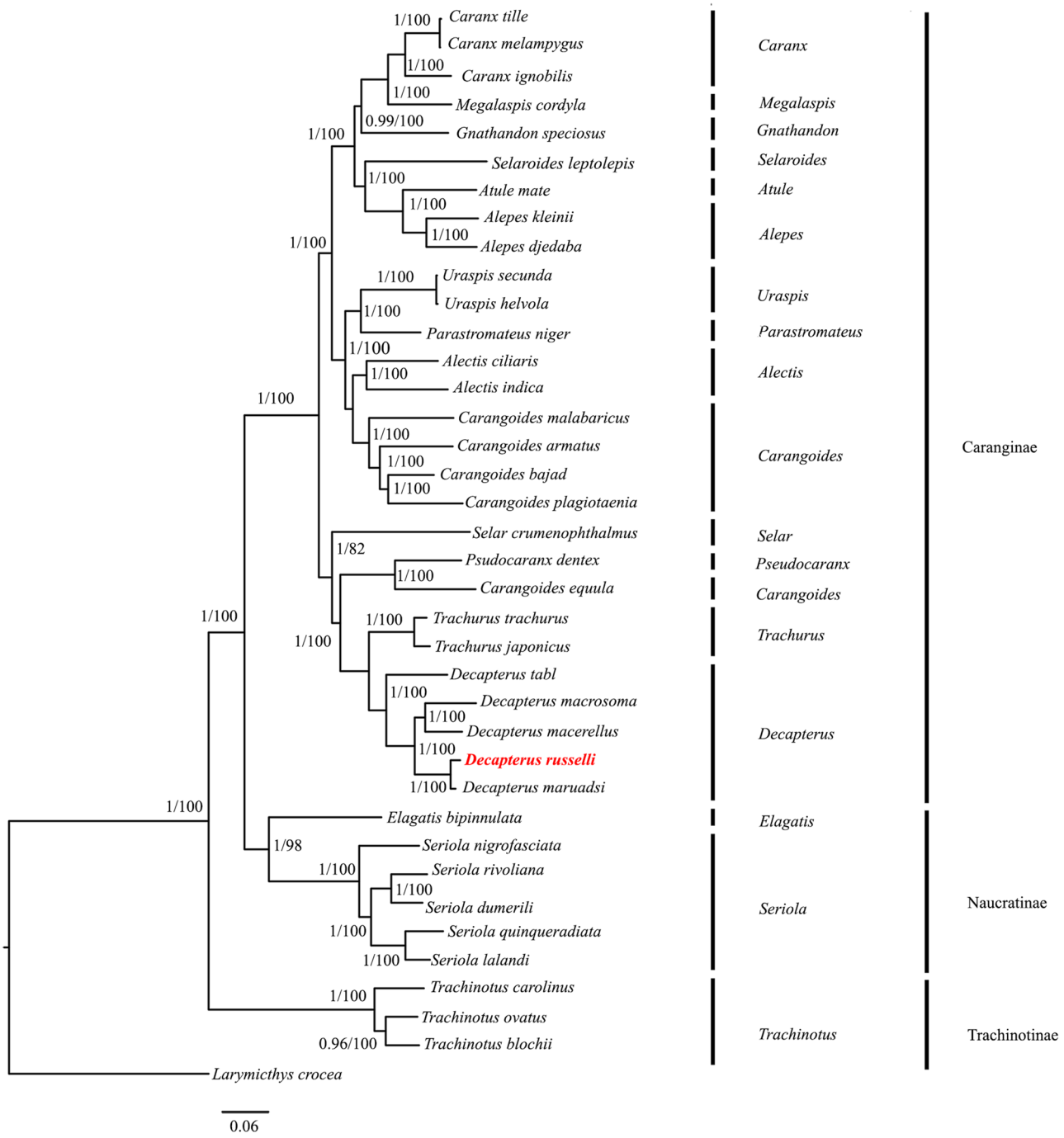


**Figure 3.** Phylogenetic tree inferred from the nucleotide sequences of 13 concatenated PCGs of 37 species of Carangidae. Bayesian posterior probabilities (left) and bootstrap support values (right) are superimposed with each node. The phylogenetic tree was generated by using MrBayes 3.2.7a[93].

conservation of its sequence suggest the functional significance of these sites. The high rate of sequence divergence in the flanking regions of conserved sequence motifs indicated the absence of typical mitochondrial control region coding constraints. The presence of evolutionarily conserved secondary structures of the conserved sequence motifs despite sequence divergence revealed the effect of selective constraints on their maintenance. The length variations in protein-coding, tRNA, and rRNA genes among Carangidae were negligible.

The phylogeny inferred from 13 gene concatenated data set resolved the monophyletic lineage of each subfamily and robustly supported the grouping (Trachinotinae + (Caranginae + Naucrautinae)) with the caveat that the subfamily Scomberoidinae could not be included as the mitogenome sequences were not available. Further, the speciation of the genus *Decapterus* occurred before the separation of Tethys Sea and *D. russelli* diverged before the mid-Pliocene warm period. Future studies involving the mitogenomic data of Scomberoidinae are required for a better understanding of the phylogenetic and evolutionary relationship between major groups

within the Carangidae. Mitogenomic phylogeny is also influenced by the habitat characteristics of each group, as habitat-specific selection and adaptation in mitochondrial coding genes has recently been reported[79]. The subfamilies Trachinotinae, Naucratinae, and Caranginae consist of species with differential habitat preferences (pelagic, benthopelagic, and reef-associated) and schooling behavior. The mutational patterns in the protein-coding regions of these fish need to be further analyzed for adaptive variation and selection and correlated with habitat characteristics to understand the forces of selection, if any.

## Materials and methods

**Sampling.** The wild specimen of *D. russelli* was collected from Kochi, Kerala (9°56′19"N 76°15′45" E) from trawl and purse seines. The species was confirmed based on morphological characters. Tissue samples were preserved in 95% ethanol for DNA extraction and further analysis. The fish sample used in this study was handled according to the guidelines for the care and use of fish in research by De Tolla et al.[80]. The protocols were approved by the ethical committee of the ICAR- Central Marine Research Institute, Kochi. These methods are also reported following ARRIVE guidelines (http://arriveguidelines.org).

**DNA extraction and PCR amplification.** Genomic DNA was isolated as previously described[81]. The extracted DNA was then eluted in T.E buffer (1X) and stored at − 20 °C for later use as a PCR template. The quality and quantity (concentration) of the isolated total DNA was checked by gel electrophoresis and NanoDrop One Spectrophotometer (Thermo Scientific, USA), respectively. The complete mitochondrial genome of *D. russelli* was determined by combining the sequences of contiguous and overlapping fragments amplified by PCR using both universal as well as custom-designed primers. Initial amplifications were performed using mitochondrial universal primer sequences (FishF1 + FishR1, 16SaRL + 16SbRH, 12S1 + 12S2, Dloop-Thr-F + Dloop-Phe-R, ATP 8.2 L8331 + CO3.2 H9236)[82–86]. The sequenced products of this reaction then served as a template for subsequent amplification. Fifteen sets of new primer pairs were designed based on the aligned complete mitogenome sequences of 36 species of carangids using Primer 3 software (http://frodo.wi.mit.edu/primer3/)[87]. The above primers were screened for potential secondary targets using Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/)[88]. PCR amplifications were performed in a Biorad T100 thermal cycler (Bio-Rad, USA). The reaction mixture included 15.6 µl sterile deionized water, 2 µl buffer (10X), 0.4 µl dNTP mix (10 mM each), 0.4 µl of each oligonucleotide (10 µM), 1 µl of template DNA (50 ng/µl) and 0.2 µl (1 unit) of Taq DNA polymerase with a total reaction volume of 20 µl. For confirmation, PCR products were electrophoresed on 1.5% agarose gel (1X TBE) and visualized using a gel documentation system (Bio-Rad, USA). The products were then sequenced completely in both directions using sanger sequencing.

**Sequence analysis.** Target sequences were assembled with the software MEGA X[89]. The identity of the *D. russelli* mitogenome was verified with other carangid mitogenomes (Supplementary Table S2) by BLAST search. These mitogenome sequences served as the reference for assembly and manual annotations. The organization and the order of the genes were initially determined using the MitoFish database[90] and later verified manually by comparison with the reference sequences. The 13 PCGs and their codon usage were summarized with MEGA X[89]. The rate of Ka/Ks substitutions in the mitogenome of Carangidae was calculated by DnaSP 6.12.03[91]. tRNA genes were first annotated by using tRNA scan SE[92] with default parameters coupled with ARWEN software[57]. The secondary structure of conserved sequence motifs of the control region was inferred using Mfold web server[93]. The complete mitogenome map of the species was graphically visualized by OrganellarGenomeDRAW [94]. The 37 mitogenomes of Carangidae were compared by using CG view comparison tool (CCT)[95] with *D. russelli* as the reference sequence. Genes were assigned by Clusters of Orthologous Groups (COG) using the CG view comparison tool (CCT) and BLAST was used to align other genomes to that of *D. russelli*.

**Phylogenetic analysis.** All available mitogenome sequences of other carangid species were retrieved from GenBank to find out the relative position of *D. russelli* among carangids and to delineate its phylogenetic relationship. *L. crocea* (GenBank accession number **NC011710**) was taken as an outgroup. The 13 PCGs were aligned using Clustal W and translated into amino acid sequences in MEGA X[57]. For the subsequent phylogenetic analysis, multiple alignments were generated for the 13 concatenated protein-coding sequences of *D. russelli* and 36 other Carangidae species. The phylogenetic relationship was inferred by constructing a tree based on Bayesian inference (BI) and maximum likelihood (ML) methods. The best model, GTR + G + I, was selected using Akaike Information Criterion (AIC). Bayesian phylogenetic analysis was performed using MrBayes[96] with the Markov Chain Monte Karlo Method (MCMC). MCMC chains were run for 10 million generations and obtained a standard deviation of split frequency less than 0.01. The ML phylogenetic tree was inferred using RaxML[97] and a consensus tree was obtained with 1000 bootstrap replicates using default parameters. The final phylogenetic tree was displayed, annotated, and embellished in FigTree software (http://tree.bio.ed.ac.uk/software/figtree/)[98].

**Divergence time estimation.** Molecular dating of 37 Carangidae species was performed with the RelTimeML method in MEGA X[99] using GTR + G + I modeling. The phylogenetic tree inferred from Bayesian analysis was imported to MEGA X to estimate divergence times. The time tree web resource (http://www.timetree.org/)[100] was used to infer calibration boundaries for a pair of taxa (minimum and maximum) and the same database was used to establish the time tree of 27 genera of carangids.

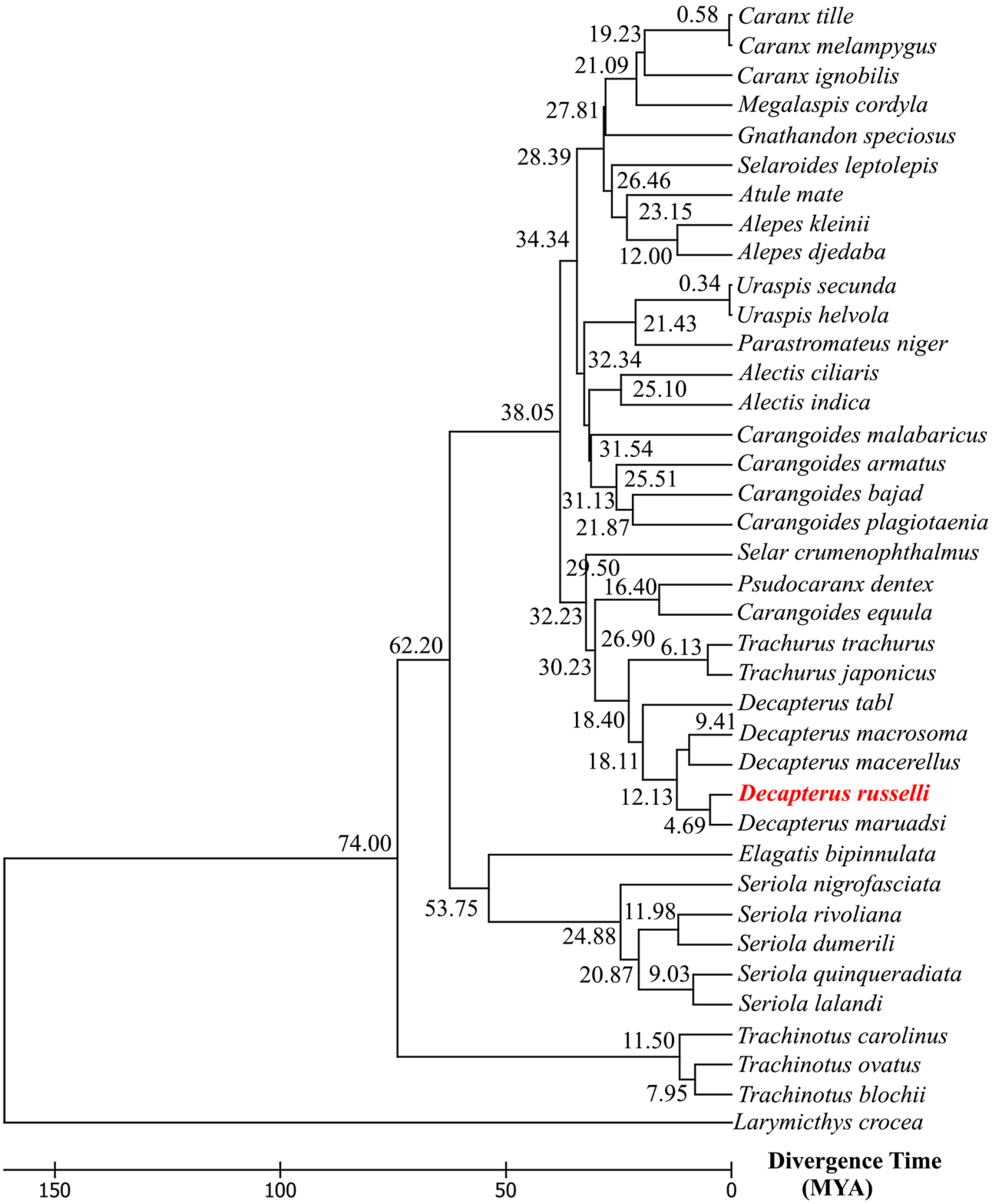


**Figure 4.** Timetree chronogram of 37 species of Carangidae for the 13 PCGs inferred from Bayesian topology. Divergence times were estimated using 32 calibrations. The red text indicates the species sequenced in this study. The Timetree was generated by using MEGA X[96].

## Data availability

All data generated or analyzed during this study is included in this published article (and its Supplementary information files).

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## Author contributions

A.J. performed the experiments, analyzed and interpreted the data and prepared the main manuscript text. S.S. conceptualized and coordinated the study and reviewed and edited the manuscript. L.P.M. and S.M. participated in the data analysis. N.R. and N.K. prepared Fig. 1. A.G. supervised the study.

## Competing interests

The authors declare no competing interests.

## Additional information

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# Genetic analyses reveal panmixia in Indian waters and population subdivision across Indian Ocean and Indo-Malay Archipelago for *Decapterus russelli*

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**The Indian Scad, *Decapterus russelli* is an important pelagic carangid widely distributed throughout the Indian Ocean and the Indo-West Pacific. Despite being widely distributed in the Indian Ocean, the information regarding genetic structuring and diversity has been lacking compared to its Indo Malay Archipelago counterparts. The present study was conducted to investigate the genetic stock structure of *D. russelli* based on mitochondrial (*Cyt b*) and nuclear (*DrAldoB1*) markers along Indian waters. The results indicated the presence of a single panmictic stock across the Indian Ocean region. High haplotype diversity associated with low nucleotide diversity suggested a population bottleneck followed by rapid population growth. Phylogenetic analysis revealed the absence of geographical clustering of lineages with the most common haplotype distributed globally. The pelagic life style, migratory capabilities, and larval dispersal may be the contributing factors to the observed spatial homogeneity of *D. russelli*. However, significant genetic differentiation was observed between the populations from Indian Ocean and Indo-Malay Archipelago. Hierarchical molecular variance analysis (AMOVA), pairwise $F_{ST}$ comparisons and SAMOVA showed existence of two distinct genetic stocks of *D. russelli* in the Indian Ocean and IMA. The observed interpopulation genetic variation was high. A plausible explanation for the genetic differentiation observed between the Indo-Malay Archipelago and the Indian Ocean regions suggest the influence of historic isolation, ocean surface currents and biotic and abiotic features of the ocean. Also, there was a significant relationship between genetic distance and geographical distance between population pairs in a manner consistent with isolation-by-distance. These resulted in the evolution of a phylogeographic break for this species between these regions. The findings of these results suggest that *D. russelli* from the Indian Ocean shall be managed in its entire area of distribution as a single stock. Further, the Indian Ocean and Indo-Malayan stocks can be managed separately.**

Understanding the population structure of any fish is of paramount importance as conservation strategies focus solely on whether the population under consideration is a single or discrete evolutionary unit. Unraveling the underlying mechanisms that lead to intra- and interspecific diversity[1], as well as historical and recent demographic processes that collectively shape the spatial pattern of genetic diversity is key to creating conservation planning[2]. The interplay between organisms and their environment in response to ecological and anthropogenic pressures[3] viz., overexploitation[4–6], habitat loss and fragmentation[7–9], climate change[10,11], biotic exchange[12,13], etc. have counterintuitive impact on the current evolutionary process, making it difficult to decipher and quantify their potential roles. In recent years, much research has focused on the genetic patterning and environmental adaptation of marine fish, finding populations that are genetically diverse[14], spatially structured[15,16] and locally adapted[17,18]. For spatially structured populations characterized by high levels of site fidelity, it is unlikely that the effects of overexploitation or habitat fragmentation at one site would have deleterious effects on neighboring

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populations[17]. On the contrary, if the populations are migratory and are highly interbreeding, one could easily manifest the effects on one population in nearby areas[19].

Many marine organisms are characterized by a large effective population size and high dispersal capacity, both as larvae and adults[20]. These traits could result in high gene flow between populations over time and space unless separated by geographic or hydrological barriers that reduce migration between areas. Interspecific differences in population biology are exhibited by several migratory species. Several studies spanning a wide range of taxa from the Indian Ocean have identified many species with high levels of genetic structure[14,21–24]. However, some other species interbreed and spread extensively, resulting in low population structure[25–29]. In *Alosa pseudoharengus*, variations in patterns of genetic structure between landlocked and anadromous populations have been observed[30]. Several species of migratory fish are reported to have experienced staggering population size declines worldwide[31–33]. Pelagic sharks (*Alopias* spp., *Carcharodon carcharias* and *Sphyrna* spp.) have declined by over 75% in the Northwest Atlantic since 1986[34]. Sporadic reports of similar declines have also been recorded for other predatory marine fishes[35–37]. Failure to halt this decline will ultimately result in the loss of these species from their current habitats and has evolutionary consequences. In addition, small populations often experience high levels of genetic drift, fixation of harmful alleles and loss of genetic diversity, eventually leading to loss of genetic integrity and negatively impacting their adaptive potential[38]. Therefore, a better understanding of a species' population structure and movement patterns is required for effective conservation and equitable management of fishery resources.

The objective of the present study was to investigate the population structure of a highly mobile pelagic fish, *Decapterus russelli*. It is widespread in the Indo-Pacific from the Red Sea and East Africa to Japan and Australia[39,40]. The Indian scad, *D. russelli* represents an important fishery resource in India and Southeast Asia[40]. It represents an important pelagic resource of India, ranking next to the Indian oil sardine[41], is an inexpensive source of animal protein[42] and is widely used as a live fish bait[39]. Indian scad mackerel inhabits at depths of 40–275 m and are most commonly found on the coast and open shores of the Indian Ocean[39]. The species has a prolonged spawning season, with different studies reporting different spawning period overlapping along different coasts of India: February–November[43], November–December[44], March–December with peak in February and March[45], December and September[46], December–March and June–October with a peak in January and September[47] on the southwest coast; December and August on the north-west coast[46]; April–August[46] and December–July, sometimes extending to August[48], November–May[49] on the east coast of India, suggesting that this species is a continuous breeder preferring to spawn only when conditions are favorable, which can vary from place to place. Ovarian development is asynchronous and the eggs are pelagic and spherical in shape with size ranging from 0.02 to 0.97 mm[50]. The exact spawning grounds have not yet been located. The species plays an important role in the proper functioning of the ecosystem, as it avoids direct competition by increasing the number and diversity of prey items as they grow. Planktonic crustaceans (*Acetes* spp., copepods and other crustaceans) formed the main diet in smaller groups[45] and were replaced by fish (*Lactarius* spp., *Myctophid* spp.) and molluscs[49,51,52] in larger groups. As a primary carnivore, they inevitably help keep the ecosystem in balance as they are a voracious feeder of planktonic crustaceans[45] and keep their population at optimal levels. They also play a crucial role in maintaining trophic ecology and the integrity of the food web. The large-scale consumption of high-energy planktonic crustaceans[45,51] makes this highly vagile species a potential link in the food chain to temporally and spatially transfer energy to subsequent trophic levels.

*D. russelli* has been characterized primarily for its biology and population dynamics[44,48,49], length–weight relationship[53], diet and feeding habits[51], reproductive biology[54–56], and population status[46,57]. Extensive studies of its geographic structure have also been carried out from the Indo-Malay Archipelago (IMA). Previous studies have reported that two populations of scad mackerel enter the Java Sea seasonally, one during the east monsoon from the Flores Sea and Makassar Straits and another during the west monsoon from the Indian Ocean and Sunda Straits[58]. In addition, it also suggested the possibility that a third population could enter the Java Sea from the South China Sea during the west monsoon. The migration and distribution of Indian scad mackerel can be ascribed to the variation in salinity associated with the monsoon cycle in the IMA[58]. Two putative lineages have been distinguished from IMA in *D. russelli* based on the sequence polymorphism of the mitochondrial cytochrome b (*Cyt b*) gene. The two lineages reportedly showed spatial heterogeneity in their distribution. Later, Rohfritsch and Borsa[40] discovered three mitochondrial lineages of *D. russelli* from IMA based on the combined result of mitochondrial and nuclear markers. One of the Perrin and Borsa[59] haplogroups appeared to consist of two separate lineages in Rohfritsch and Borsa[40]. They could also observe the co-occurrence of some mitochondrial lineages, indicating recent secondary contact between once isolated populations or admixture in samples of cryptic species[60].

Genetic information on this species from the Indian Ocean is scarce apart from a study of its complete mitochondrial genome[42]. Furthermore, with the exception of one study using truss morphometric analysis[24], *D. russelli* from the Indian Ocean are less studied (particularly using molecular markers) than their counterparts in the Indo-Malay Archipelago. Sen et al.[24] used 23 variables to distinguish between populations and identified separate stocks on the east and west coasts of India. The genetic basis for this observed phenotypic discreteness needs to be further elucidated in order to derive effective management recommendations for the species in different geographic areas in the Indian Ocean. Therefore, we examined the genetic population structure of Indian scad mackerel collected from five locations using mitochondrial and nuclear markers. In addition, to detect any genetic breaks between the populations of *D. russelli*, we performed a comparative study by expanding the geographic area to IMA. Since the species has high economic value and is widely harvested, the genetic data of such a species will provide adequate information to implement effective management strategies for the sustainable use of fishery resources.

# Results

## Genetic diversity analysis

*Mitochondrial DNA (Cyt b) analysis*

The *Cyt b* gene sequences of a total of 125 individuals sampled from five locations along the Indian coast were sequenced. After alignment and editing of ambiguous sequences, a final sequence length of 933 bp was obtained. The aligned dataset revealed an overall of 58/933 (6.21%) variable sites where 14/993 sites (1.50%) were parsimony-informative with 38 haplotypes. All haplotypes were deposited in GenBank under accession numbers OR188721 to OR188758. Furthermore, 34 (89.47%) haplotypes were singletons and 4 (10.52%) were shared between populations. The average base composition of the sequences was 23.2% A, 27.5% T, 15.4% G and 33.9% C with a higher A/T content compared to G/C. The transition to transversion ratio was 3:1. A detailed evaluation revealed 34 nonsynonymous mutations with 33 amino acid substitutions. The overall haplotype and nucleotide diversity was 0.6723 and 0.00167, respectively. The descriptive statistics of each population from the Indian Ocean are presented in Supplementary Table S1.

The previously reported IMA sequences were also included in the study[40] bringing the total number of sequences to 583. We trimmed our sequences to match those retrieved from GenBank resulting in a final length of 307 bp. These data were used for further analysis. Altogether, the dataset revealed 30 variable sites, of which 14 were parsimony informative and 16 singleton variable sites with 27 haplotypes (Table 1) with an overall haplotype diversity (h) of 0.49684 and a nucleotide diversity ($\pi$) of 0.00593. Twenty out of the 27 haplotypes were found exclusively in the IMA, while the remaining 7 were discovered from the Indian Ocean. Out of a total of 27 unique haplotypes, only 8 (30%) were found in more than one region, with the rest being region-specific. In comparison, haplotype and nucleotide diversity in the IMA population ($h = 0.25029$, $\pi = 0.00364$) were higher than that in the Indian Ocean population ($h = 0.10916$, $\pi = 0.00078$). Furthermore, haplotype and nucleotide diversity in IMA ranged from 0.00 (Arafura) to 0.48 (Makassar) and from 0.00 (Arafura) to 0.00951 (Makassar), respectively (Table 2). The haplotype and nucleotide diversity in the Indian Ocean population ranged from 0.00 (Cochin) to 0.15667 (Mangalore and Veraval) and from 0.00 (Cochin) to 0.00261 (Mangalore), respectively. The IMA populations consisted mostly of singletons (13/20–65%) (Table 1). The average base composition of the entire sequences was 21.8% A, 31.2% T, 15.6% G, and 31.3% C. The ratio of transition to transversion was 4:1. The entire dataset revealed seven non-synonymous mutations that resulted in seven amino acid changes. It has been observed that the haplotypes shared by the IMA population are not detected in the Indian Ocean population and vice versa.

The genetic diversity or distance values were converted to percentages and most of the genetic diversity within the population was 0%, except for Toli-Toli and Makassar which have a genetic distance of 1% (Supplementary Table S2). When populations with little genetic distance were excluded, genetic diversity between populations ranged from 1 to 2%. Makassar and Toli-Toli from the IMA differed the most of all, with at least 1% genetic distance from the remaining populations. The genetic distance of 2% was mainly observed between Indian Ocean and IMA populations (Mangalore and Toli-Toli; Mangalore and Makassar; Puri and Toli-Toli). Furthermore, within the IMA, a 2% divergence was observed between the populations of Toli-Toli and Tambalen (western region of the IMA). In addition, all other Indian Ocean populations are genetically separated from the IMA by 1%. However, subtle genetic differences were observed between the Indian Ocean populations and also between the populations from the western region of the IMA. The majority of $F_{ST}$ values observed in the IMA were significantly different ($p < 0.05$) (Supplementary Table S2). In addition, significant differences in $F_{ST}$ values ($p < 0.05$) were also observed between all IMA and Indian Ocean populations. However, non-significant values ($p > 0.05$) were found between populations from the Indian Ocean.

*Nuclear DNA (DrAldoB1) analysis*

A total of 125 individuals (250 alleles) of *D. russelli* from the Indian Ocean were assayed for 212 bp of intron 1 of the *Aldolase b* (*DrAldoB1*) gene. 36 unique alleles were identified of which 33 alleles were exclusively from the Indian Ocean (Table 3) and the remaining three were from IMA (IMA sequences were retrieved from NCBI-GenBank). One allele was common to all five geographical locations in the Indian Ocean (Table 3). The sequences were deposited in GenBank under accession numbers OR546246 to OR546278. Overall, the haplotype diversity was 0.3786 and nucleotide diversity was 0.01. For each population, the haplotype diversity and nucleotide diversity ranged from 0.153 (Veraval) to 0.628 (Chennai) and 0.001(Veraval) to 0.022 (Chennai) respectively (Supplementary Table S3). We observed 72 polymorphic sites out of 212 bp sequence of *DrAldoB1* of which 11 sites were the sites with more than two variants. The observed intrapopulation genetic diversity varied from 0.1% (Veraval) to 1% (Puri and Mangalore). The inter population genetic distance was negligible between the Indian Ocean populations (Supplementary Table S4). Whereas, all of the Indian Ocean population displayed a higher genetic distance between IMA, ranging from 1.3% (Veraval) to 1.9% (Puri) (Supplementary Table S4. The observed global $F_{ST}$ value was low (0.052). Pairwise $F_{ST}$ analysis revealed a lack of genetic structure in the areas studied from the Indian Ocean and was not significant ($p > 0.05$) (Supplementary Table S4). However, significant $F_{ST}$ values ($p < 0.05$) were obtained between IMA and Indian Ocean populations.

## Population structure

*Within Indian Ocean analysis*

AMOVA analysis was performed on the 5 populations from the Indian Ocean subdivided into 1-4 gene pools. In all analyses, the overall levels of genetic differentiation between coasts were non-significant ($p > 0.05$) even for the highest variance ($F_{CT} = 0.01090$, $p > 0.05$) with a global $F_{ST}$ of 0.01030. The genetic differentiation among population within groups/coast was − 0.06% ($F_{SC} = -0.00061$, $p > 0.05$). 98.97% percentage of the total variation of *D. russelli* from the Indian Ocean is contributed by the genetic differences among the total populations.

| Region | East coast of India | | West coast of India | | | Indo Malay Archipelago | | | | | | | | | | | Haplotype frequency (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population | Chennai | Puri | Kochi | Mangalore | Veraval | Kelang | Carita | Labuan | Tambelan | Pekalongan | Kinabalu | Sandakan | Toli-Toli | Makassar | Arafura | Total | Total |
| Haplotype 1 | | | | | | 1 | | | | | | | | | | 1 | 0.17 |
| Haplotype 2 | | | | | | | | | | | | 1 | | | | 1 | 0.17 |
| Haplotype 3 | | | | | | | | | | | | 1 | | | | 1 | 0.17 |
| Haplotype 4 | | | | | | | | | | | | | 22 | 14 | | 36 | 6.20 |
| Haplotype 5 | | | | | | | | | | 1 | | | | | | 1 | 0.17 |
| Haplotype 6 | | | | | | | 1 | 2 | | | | | | | | 3 | 0.51 |
| Haplotype 7 | | | | | | | | 1 | | | | | | | | 1 | 0.17 |
| Haplotype 8 | | | | | | | | | 1 | | 1 | | | | | 2 | 0.34 |
| Haplotype 9 | | | | | | | 1 | 1 | 1 | | 1 | | | | | 4 | 0.70 |
| Haplotype 10 | | | | | | | | | 1 | | | | | | | 1 | 0.17 |
| Haplotype 11 | | | | | | | | | | 1 | | | | | | 1 | 0.17 |
| Haplotype 12 | | | | | | | | | | 1 | | | | | | 1 | 0.17 |
| Haplotype 13 | | | | | | | | | | | | 1 | | | | 1 | 0.17 |
| Haplotype 14 | | | | | | | 1 | | | | | | | | | 1 | 0.17 |
| Haplotype 15 | | | | | | | | | | | | 1 | | | | 1 | 0.17 |
| Haplotype 16 | | | | | | | | 1 | | | | | | | | 1 | 0.17 |
| Haplotype 17 | | | | | | | | | 1 | | | | | | | 1 | 0.17 |
| Haplotype 18 | | | | | | 1 | 1 | | | | | 1 | | | | 3 | 0.51 |
| Haplotype 19 | | | | | | 10 | 38 | 72 | 48 | 64 | 35 | 61 | 8 | 8 | 51 | 395 | 67.75 |
| Haplotype 20 | | | | | | | | 2 | | | | | | | | 2 | 0.34 |
| Haplotype 21 | 24 | 23 | 25 | 23 | 23 | | | | | | | | | | | 118 | 20.24 |
| Haplotype 22 | | | | 1 | | | | | | | | | | | | 1 | 0.17 |
| Haplotype 23 | | | | 1 | | | | | | | | | | | | 1 | 0.17 |
| Haplotype 24 | | | | | 1 | | | | | | | | | | | 1 | 0.17 |
| Haplotype 25 | 1 | | | | 1 | | | | | | | | | | | 2 | 0.34 |
| Haplotype 26 | | 1 | | | | | | | | | | | | | | 1 | 0.17 |
| Haplotype 27 | | 1 | | | | | | | | | | | | | | 1 | 0.17 |
| Total | 25 | 25 | 25 | 25 | 25 | 12 | 42 | 79 | 52 | 67 | 37 | 66 | 30 | 22 | 51 | 583 | |

**Table 1.** Distribution of haplotypes for 15 populations of *D. russelli* based on *Cyt b* gene sequence data.

| | Indian Ocean | | | | | Non-Indian Ocean | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | East Coast | | West Coast | | | IMA | | | | | | | | | |
| | Chennai | Puri | Cochin | Mangalore | Veraval | Kelang | Carita | Labuan | Tambelan | Pekalongan | Kinabalu | Sandakan | Toli-Toli | Makassar | Arafura |
| N | 25 | 25 | 25 | 25 | 25 | 12 | 42 | 79 | 52 | 67 | 37 | 66 | 30 | 22 | 51 |
| Number of haplotypes | 2 | 3 | 1 | 3 | 3 | 3 | 5 | 6 | 5 | 4 | 3 | 6 | 2 | 2 | 1 |
| Number of polymorphic sites | 1 | 2 | 0 | 10 | 2 | 2 | 8 | 9 | 6 | 10 | 4 | 5 | 6 | 6 | 0 |
| Haplotype diversity, Hd | 0.0800 | 0.15667 | 0.00 | 0.15667 | 0.15667 | 0.31818 | 0.18351 | 0.16975 | 0.14932 | 0.08820 | 0.10661 | 0.14658 | 0.440460 | 0.48485 | 0.000 |
| Nucleotide diversity | 0.00026 | 0.00052 | 0.00 | 0.00261 | 0.00052 | 0.00109 | 0.00140 | 0.00177 | 0.00087 | 0.00098 | 0.00070 | 0.00068 | 0.00791 | 0.000948 | 0.000 |
| Tajimas D | −1.15753 | −1.51406 | 0.00000 | −2.30550 | −1.51406 | −1.45138 | −2.17642 | −1.81405 | −2.01660 | −2.33128 | −1.88391 | −1.83587 | 1.72038 | 2.37829 | 0.00000 |
| Fu's Fs | −1.06131 | −2.12796 | 0.00000 | 0.91456 | −2.12796 | −1.32484 | −2.61585 | −2.49722 | −3.82287 | −1.84208 | −1.37648 | −6.31047 | 7.19054 | 7.43462 | 0.00000 |
| *Hri* index | 0.71200 | 0.49861 | 0.00000 | 0.60492 | 0.49861 | 0.22658 | 0.48821 | 0.58186 | 0.60829 | 0.78190 | 0.71515 | 0.59213 | 0.68190 | 0.73554 | 0.00000 |

**Table 2.** Sample size, population genetic statistics and historical demographic analysis of 15 *D. russelli* populations based on mitochondrial *Cyt b* gene sequences.

| Alleles | Population | | | | | Total |
|---|---|---|---|---|---|---|
| | Chennai | Puri | Cochin | Mangalore | Veraval | |
| 1 | | | | | 2 | 2 |
| 2 | | | | | 2 | 2 |
| 3 | 30 | 46 | 39 | 36 | 46 | 197 |
| 4 | | 1 | | | | 1 |
| 5 | | 1 | | | | 1 |
| 6 | | 1 | | | | 1 |
| 7 | | 1 | | | | 1 |
| 8 | | | | 2 | | 2 |
| 9 | | | | 2 | | 2 |
| 10 | | | | 1 | | 1 |
| 11 | | | | 1 | | 1 |
| 12 | | | | 2 | | 2 |
| 13 | | | | 2 | | 2 |
| 14 | | | | 2 | | 2 |
| 15 | | | | 2 | | 2 |
| 16 | 6 | | | | | 6 |
| 17 | 1 | | | | | 1 |
| 18 | 1 | | | | | 1 |
| 19 | 3 | | | | | 3 |
| 20 | 1 | | | | | 1 |
| 21 | 2 | | | | | 2 |
| 22 | 2 | | | | | 2 |
| 23 | 1 | | | | | 1 |
| 24 | 1 | | | | | 1 |
| 25 | 1 | | | | | 1 |
| 26 | 1 | | | | | 1 |
| 27 | | | 1 | | | 1 |
| 28 | | | 1 | | | 1 |
| 29 | | | 2 | | | 2 |
| 30 | | | 2 | | | 2 |
| 31 | | | 3 | | | 3 |
| 32 | | | 1 | | | 1 |
| 33 | | | 1 | | | 1 |
| Total | 50 | 50 | 50 | 50 | 50 | 250 |

**Table 3.** Unique alleles of *Aldolase b* detected in *D. russelli* population from the Indian Ocean.

Furthermore, AMOVA analysis undertaken with nuclear markers also demonstrated the lack of significant genetic structure ($p > 0.05$) in *D. russelli* (Supplementary Table S5). This result indicated no subdivision or structuring within the tested populations. Additional genetic differentiation analysis revealed that estimates based on $H_{ST}$ (0.006), $N_{ST}$ (0.002) and $K_{ST}^{*}$ (0.001) were all very low and none of them were significant ($p > 0.05$). This also indicates a high level of gene flow among the sampled *D. russelli* populations from Indian Ocean (Nm = 207.34 for haplotype-based statistics and Nm = 83.97 for sequenced based statistics). On the other hand, the IMA population, divided into two geographical groups, revealed significant genetic structuring at all levels (Table 4). The mantel test showed no significant correlation between genetic distance (pairwise $F_{ST}$ values) and geographical distance ($r = 0.063$, $p > 0.05$) among the tested population (Supplementary Fig. S1). Overall, 4 and 3 populations out of 5 were significant with negative values of Tajimas' D and Fu's Fs (Supplementary Table S1) suggesting historical population expansion at these sites associated with an excess of recent mutations or rare alleles[61]. The Harpending raggedness index (*Hri* = 0.46, SSD = 0.03, P = 0.02) corroborated these results. Mismatch analysis revealed a unimodal distribution providing strong evidence for a sudden population expansion (Supplementary Fig. S2). The minimum spanning network (MSN) produced a reticulation of 38 haplotypes (Supplementary Fig. S3). One haplotype was common to all geographical locations. The common haplotypes within the network are inferred to be ancestral, whereas the tip haplotypes may be derived or descended from ancestral (internal) haplotypes[62]. Thus, the occurrence of star like pattern radiating from these major haplotypes suggests that the *D. russelli* population has undergone a recent demographic expansion. Phylogenetic analysis based on mitochondrial and nuclear markers revealed no obvious phylogeographic pattern separating the 5 samples of *D. russelli* (Supplementary Fig. S4; Supplementary Fig. S5).

| Hierarchical level | Variation (%) | F-statistic | P value |
|---|---|---|---|
| Among IO | | | |
| Among regions ($F_{CT}$) | 1.09 | 0.01090 | ns |
| Among populations within region ($F_{SC}$) | − 0.06 | − 0.00061 | ns |
| Among individuals within populations ($F_{ST}$) | 98.97 | 0.01030 | ns |
| Among IMA | | | |
| Among regions ($F_{CT}$) | 83.29 | 0.83288 | * |
| Among populations within region ($F_{SC}$) | − 0.08 | − 0.00464 | * |
| Among individuals within populations ($F_{ST}$) | 16.79 | 0.83211 | * |
| Between IO and IMA | | | |
| Among regions ($F_{CT}$) | 72.09 | 0.72094 | * |
| Among populations within region ($F_{SC}$) | 14.14 | 0.50668 | * |
| Among individuals within populations ($F_{ST}$) | 13.77 | 0.86233 | * |

**Table 4.** Results of the analysis of molecular variance (AMOVA) for *D. russelli* showing F-statistics analysis of mitochondrial *Cyt b. IMA* Indo-Malay Archipelago, *IO* Indian Ocean. *ns* not significant ($P > 0.05$). *$P < 0.01$.

*Between Indian Ocean and IMA analysis*

The AMOVA analysis was also performed on the 15 populations (10 from the IMA and 5 from the Indian Ocean) that were subdivided into two geographical groups (Group 1: Indian Ocean; Group 2: IMA) to determine whether there is any genetic structuring over the geographical area. AMOVA analysis revealed that genetic differentiation was significant at all levels (Table 4), with 72.09% of the total variation attributed to variation among regions/groups, implying significant differentiation across geographical areas. The analysis was also repeated with the IMA populations divided into three geographical locations. The test is to discover whether the genetic differentiation was visible at the finer spatial scales across the IMA. Thus, the sample was divided into 4 groups (Group 1: Indian Ocean; Group 2: Makassar strait/Sulawesi Sea; Group 3: Arafura Sea; and Group 4: western region of the Indo-Malay Archipelago). The analysis generated a non-significant albeit low $F_{SC}$ value, whereas significant differentiation among groups ($F_{CT} = 0.8267$, $p < 0.05$) was observed accounting for the highest percentage (82.77) of the total variation. The analysis was repeated splitting the Indian Ocean population into two groups based on coast, bringing the total group to five. The groups were, Group 1: Western Indian Ocean; Group 2: Eastern Indian Ocean; Group 3: Makassar strait/Sulawesi Sea; Group 4: Arafura Sea; Group 5: western region of the IMA. It produced similar outcomes as the previous group of four. In addition, genetic differentiation estimates were analyzed by dividing the whole population into two: Indian Ocean and IMA. All values generated ($H_{ST} = 0.60$, $N_{ST} = 0.79$ and $K_{ST}^{*} = 0.54$) were high and significant. *DrAldoB1* also revealed significant genetic structuring ($p < 0.05$) between IMA and Indian Ocean populations (Supplementary Table S5).

The result from the SAMOVA was consistent with other analyses, with $k = 2$ showing the highest $F_{CT}$ value ($F_{CT} = 0.8362$, $p < 0.05$), clearly demonstrating the existence of two genetically distinct *D. russelli* stocks among the populations under study, namely the IMA and Indian Ocean populations. The mantel test showed a significant correlation between genetic differentiation (pairwise $F_{ST}$) and geographical distance ($r = 0.11$, $p < 0.05$) among the tested population.

## Phylogenetic relationship

The Bayesian phylogenetic tree revealed three mitochondrial lineages (Fig. 1). The two lineages were haplogroups M and A formerly identified by Perrin and Borsa[59]. Two distinct mitochondrial lineages with heterogeneous geographic distribution were found for Toli-Toli, Pekalogan, and Labuan. The third lineage consisted of populations from the Indian Ocean. In addition, the Perrin and Borsa's[59] M haplogroups formed a sister lineage with the populations from the Indian Ocean. The two major lineages were observed to be separated by an average nucleotide divergence of 2%. Haplogroup M and the Indian Ocean population were separated from haplogroup A by an average nucleotide difference of 2.2% and 1.8% respectively, when the three lineages were considered. In addition, the haplogroup M and the Indian Ocean population are separated by a genetic divergence of 2.2.%.

The Bayesian phylogenetic tree produced from *DrAldoB1* formed 2 lineages representing an Indian Ocean lineage and an IMA lineage (Fig. 2). These two lineages were observed to be separated by an average nucleotide divergence of 1.6%.

## Haplotype network diagram

The haplotype network diagram (Fig. 3) derived from partial sequences of the mitochondrial *Cyt b* gene, delineated three major clusters similar to the phylogenetic tree: haplogroup A, haplogroup M and Indian Ocean populations. The rarer haplotypes from IMA, *h1*, *h2* and *i* comprising the M haplogroup formed a distinct cluster separated from haplotype *M*, the most frequent haplotype in the haplogroup M by 4 genetic mutations, supporting the finding of Perrin and Borsa[59]. The haplogroup M has been separated from haplogroup A by 6 mutational steps. The cluster formed by the Indian Ocean population was separated from haplogroups A and M by 8 and 4 mutational steps, respectively.

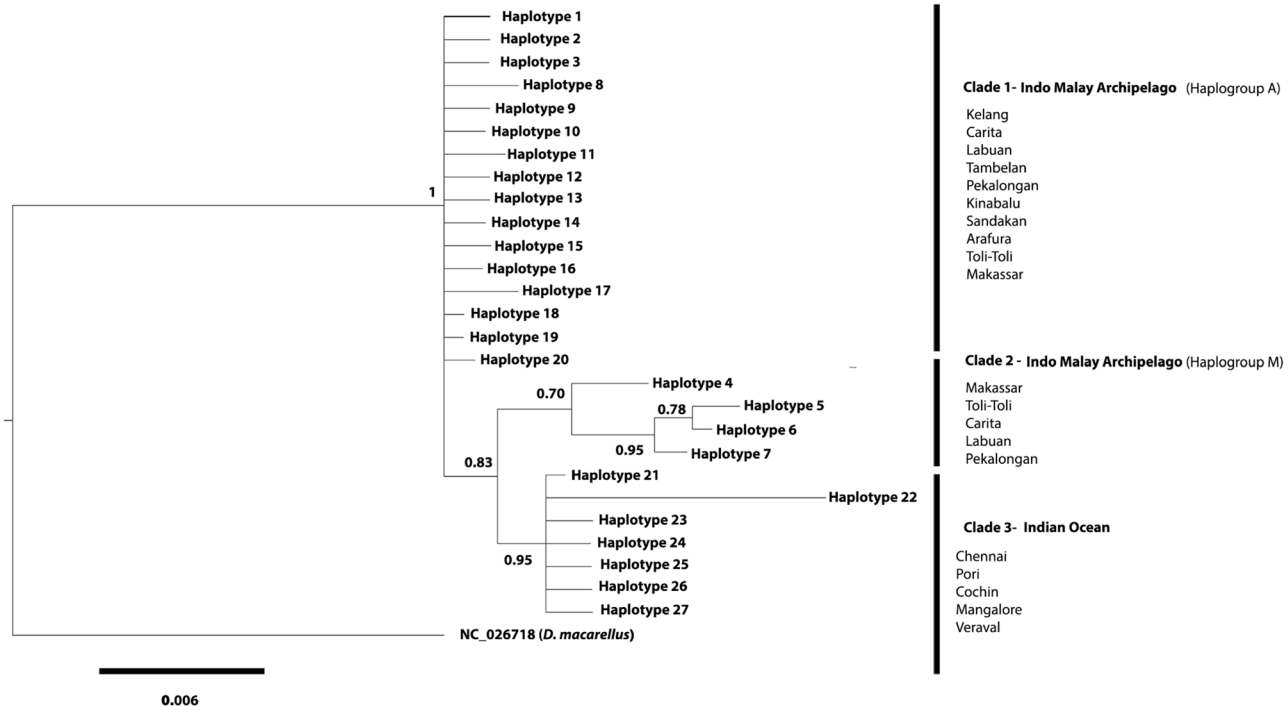


**Figure 1.** Bayesian inference phylogenetic relationships among 27 mtDNA *Cyt b* haplotypes in *D. russelli*. Bayesian posterior probabilities are superimposed with each node. The phylogenetic tree was generated using MrBayes 3.2.7.

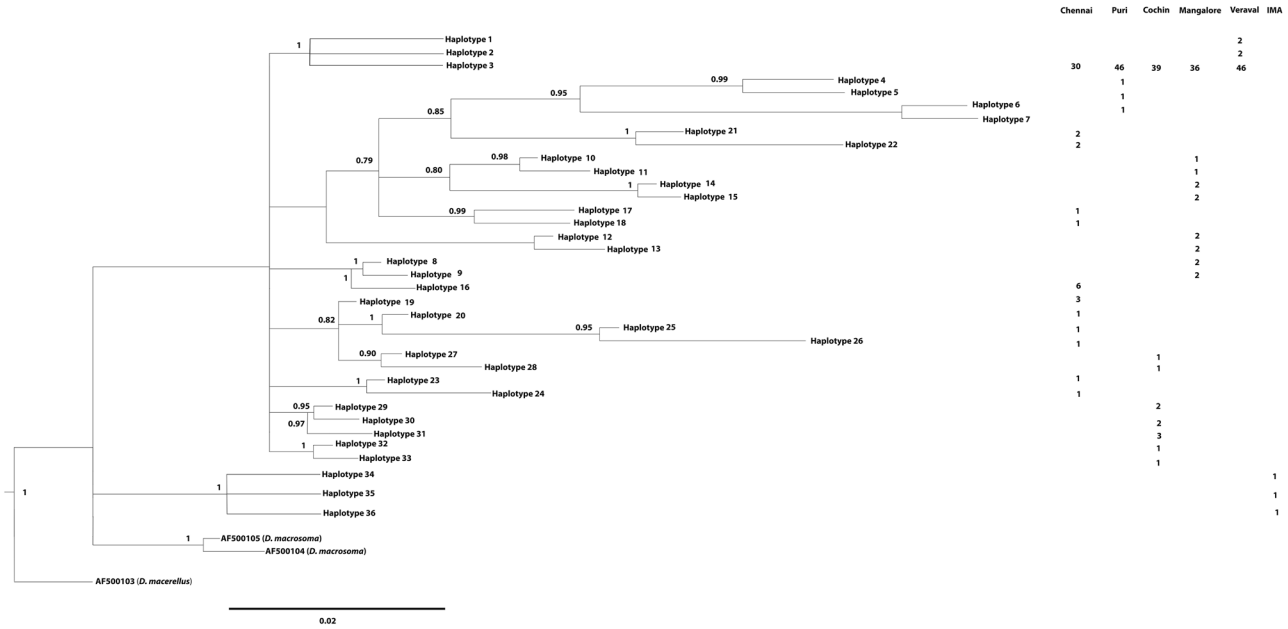


**Figure 2.** Bayesian Inference phylogenetic relationships among 36 alleles of Intron 1 partial sequences of *Aldolase b* gene of *D. russelli*. Bayesian posterior probabilities are superimposed with each node. The phylogenetic tree was generated using MrBayes 3.2.7.

## Demographic history

Overall, the 11 and 10 populations out of the total populations tested were significant with negative Tajimas D and Fu's Fs (Table 2) values respectively, indicating a recent historical directional selection (selective sweep) or population expansion[63] at these sites. In addition, the Harpending Raggedness index, $Hri = 0.5$, SSD = 0.04 ($p = 0.2$), corroborated this finding with the recent population growth hypothesis[64]. Furthermore, based on the mismatch distribution of the combined population, past population demography of *D. russelli* revealed three highly divergent peaks, representing the three lineages (Supplementary Fig. S2). A bimodal mismatch distribution

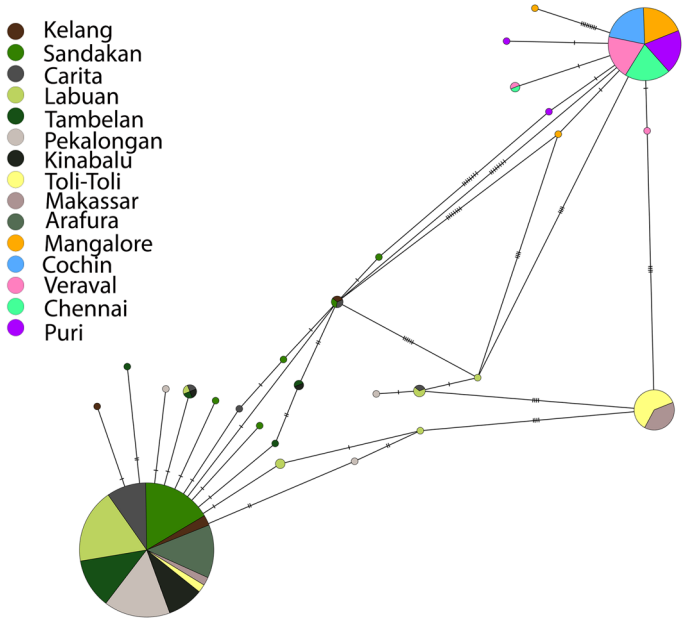


**Figure 3.** Minimum spanning network inferred from mitochondrial *Cyt b*. Colored circles represent different regions (refer to legend). The network diagram of all haplotypes was constructed based on median joining calculations in PopARTv1.7.

was found in the IMA lineage 2 after additional independent analysis of the three lineages. However, further comprehensive studies from the IMA are needed to confirm this preliminary finding. The IMA lineage 1 and the Indian Ocean (IO) lineage on the other hand, has a unimodal distribution indicating a rapid expansion of its population.

The time of demographic and spatial expansion for the major lineages 1 (IMA lineage 1) and 2 (IMA lineage 2 + IO lineage) using the formula $\tau = 2\ \mu t$ are estimated to occur 8,58,934–4,46,254 years ago and 4,57,680–4,46,254 years ago respectively. Therefore, the spatial expansion coincided with the demographic expansion. Furthermore, the spatial and demographic expansion of IO lineage occurred between 6,11,285–3,17,589 years ago. In addition, the demographic expansion of lineage 2 (IMA lineage 2) occurred between 7,93,1596–3,17,589 years ago, whereas the spatial expansion of its population was between 3,66,771–1,90,553 years ago. Therefore, this lineage experienced demographic expansion following spatial expansion.

## Discussion

Genetic data based on mitochondrial and nuclear markers revealed little divergence among the five populations analyzed, suggesting the existence of panmictic populations of Indian Scad, *D. russelli* from Indian waters. The lack of significant genetic differentiation between populations as indicated by AMOVA analysis ($F_{ST} > 0.05$) suggests that *D. russelli* populations can be managed as a single stock in Indian waters. These findings contradicted the previous study which suggested the presence of two distinct stocks of *D. russelli* along the east and west coasts of India[24]. The previous study was conducted based on truss morphometry over a small geographical area, with opportunistically collected individuals over a short period of time probably resulting in a potential artifact of population subdivision. Morphometric and meristic characters are particularly vulnerable to environmental influences and are considerably impacted once the species is exposed to environmental stressors. These environmental stressors act as selection pressures affecting the structure and function of organisms, reducing their Neo-Darwinian fitness[65]. Environmental conditions such as currents or water masses and various stressors, including biotic and abiotic factors such as food availability, salinity and temperature are subject to temporal and spatial variability and affect the morphometry of the species at each site at any given time. Although it is widely acknowledged that the environment plays a role in shaping the phenotype, there are additional important factors influencing phenotypic variation that are being investigated at the molecular level[66]. Thus, the variations reported by Sen et al.[24] in the specimens from the Arabian Sea and Bay of Bengal can be proposed as a consequence of phenotypic plasticity in response to temporal variations in environmental and oceanographic parameters.

In this study, the high haplotype diversity, Hd (values ranging from 0.43 to 0.90) coupled with low nucleotide diversity, $\pi$ (ranging from 0.0007 to 0.002) observed in all populations was concordant with previous reports of several pelagic marine species from the Indian Ocean. Previous studies from the Indian Ocean include *Euthynnus affinis* (Hd = 0.9, $\pi = 0.01$)[67], *Xiphias gladius* (Hd = 0.88 $\pi = 0.002$)[68], *Rastrelliger kanagurta* (Hd = 0.95 $\pi = 0.008$)[69] and *Thunnus obesus* (Hd = 0.99 $\pi = 0.04$)[27]. High haplotypic diversity within populations can be maintained for a variety of reasons, including large population sizes, environmental variation, and life-history factors that favor rapid population growth[70,71]. The high-level haplotype diversity observed in this study may be due to the large

effective population size and wide distribution as suggested for other pelagic fishes[72,73]. *D. russelli* is also the most abundant carangid in Indian waters[41].

It has been proposed that population history of marine species can be classified into four categories based on different values of haplotype diversity (Hd) and nucleotide diversity (π) of mt DNA gene sequences[74]. High Hd values and low π values observed in the present study indicate a recent population expansion from a small effective population size, i.e. a rapid population increase is seen in *D. russelli* population after a bottleneck event with mutation accumulation (second category; Grant and Bowen[74]). The unimodal mismatch analysis, the significant negative Fu's Fs and Tajima's D values, non-significant deviation from the sum of squared deviations (SSD) and the Harpending's raggedness index (*Hri*) were also consistent in supporting the occurrence of population expansion of *D. russelli* in Indian waters.

Marine fishes are reported to have strong intraspecific gene flow than freshwater fishes and hence reduced genetic structure[75]. This is probably because the marine environment imposes fewer barriers to species dispersal. In particular, there are reports of low levels of genetic differentiation in species with a pelagic life history both within[27,67–69] and between the oceans[25,68,76], largely due to the existence of a continuous circumglobal pelagic environment and the presence of a wide range of suitable spawning grounds. One of the possible explanations for the observed lack of population subdivision in *D. russelli* samples includes seasonal fluctuations in water circulation caused by the monsoon currents in the Indian Ocean. During the northeast monsoon, the flow of the upper ocean is directed westward towards the Arabian sea. The flow direction changes during the southwest monsoon, flowing eastward from Somalia towards the Bay of Bengal[77]. This seasonal reversal of monsoon currents may homogenize *D. russelli* populations in Indian waters. In addition, the Indian Ocean is divided into two hemispheres by the equator, resulting in the well-known marine bioecological impacts determined by latitudes[68]. Furthermore, this ocean is characterized by the westward South Equatorial Current (SEC)[78] and hydrochemical south Tropical Front which separate the two large oligotrophic areas—Indian Monsoon Gyre Province (MONS) in the north and the Indian South Subtropical Gyre Province (ISSG)[79]. However, these physical and ecological divisions have little impact on the genetic structure of *D. russelli* in contrast to the Indo Malay Archipelago counterparts. In addition, the life history traits of *D. russelli* like long distance migration[58], fast growth rate[53] and high fecundity[55] contribute to a high level of gene flow, which in turn led to the low level of genetic subdivision in this species. Furthermore, the phylogenetic groups that were evident from the Bayesian analysis of both *Cyt b* and *DrAldoB1* genes data of *D. russelli* revealed the absence of geographical clustering of lineages among the Indian Ocean populations. Moreover, the haplotypes from all samples were interspersed throughout the tree.

Analysis of mitochondrial *Cyt b* and *DrAldoB1* sequences indicated strong isolation between the Indian Ocean and IMA with significant level of genetic differentiation. The Indian Ocean is impacted by climatic variability on a larger scale[77]. It displays a variety of climate variability modalities, most of which are associated with the robust seasonal cycle ranging from intra-seasonal to inter-annual and even longer time scales. It differs from the other oceans in a number of climatically important ways[78]—first, the Asian landmass blocks the northern entrance to the Indian Ocean, preventing heat export to the north. Second, the strongest monsoon on earth originates from the Asian continent. These monsoonal winds cause significant seasonal variations in ocean currents including annual reversals. In addition, unlike other oceans, the Indian Ocean does not experience consistent equatorial easterlies, which prevents it from experiencing climatological equatorial upwelling. Finally, the Indian Ocean has the Indonesian Throughflow (ITF), a low-latitude exchange route with the Pacific. The central Indo-West Pacific region is geographically and hydrologically complex and possesses the world's largest marine diversity hotspot[80]. The region experienced major changes to its shorelines during the Pleistocene[81,82]. The shallow seas of the Sunda shelf and Sahul shelf (Arafura Sea) were above the current sea level during the Pleistocene. Land barriers are also being built to separate the South China Sea from the Indian Ocean at the southernmost point and from the Sulu Sea to the east. The repeated fluctuation in sea level associated with the ice ages may be the reason for the geographic isolation of several inland seas, leading to the genetic differentiation of the species they harbored. This is believed to be the reason for the geographic discontinuity observed in many marine species between the Indian and Pacific Oceans and also for the greater species diversity observed in the Indo-Malay region[81]. These environmental factors together with the emergence of land barriers to movement between ocean basins appear to be the strongest factor for the observed genetic heterogeneity of *D. russelli* populations between the Indian Ocean and the IMA. This may also be responsible for shaping the modern genetic structure of many species that inhabit this region as we see it today[81–85].

Three phylogenetic groups were evident in the *Cyt b* data for *D. russelli*. The tree showed evidence for the existence of distinct geographical lineages present among IMA and between IMA and Indian Ocean populations. All IMA sites grouped in Clades 1 and 2 correspond to the haplotypes A and M recognized by Perrin and Borsa[59] and Rohfritsch and Borsa[40] with an obvious pattern of geographic structuring. In clade 2, one lineage is consists entirely of Makassar and Toli-Toli individuals (haplotype M) and the next lineage encompasses haplotypes *h1, h2 and i* as in Perrin and Borsa[59] and Rohfritsch and Borsa[40]. Clade 3 consisted entirely of Indian Ocean individuals. The previous reports of the co-occurrence of separate mitochondrial lineages in some samples and their heterogeneous geographical distribution (western populations and easternmost populations)[40,59] were confirmed in this study. Due to the high connectivity of the seas in the Indo-Malay Archipelago, one might anticipate a high level of gene flow between populations. However, seasonal variations in the surface currents in the western part of the archipelago in response to the typical monsoon cycle may cause changes in water circulation and salinity, which can affect the migration patterns of adult pelagic species and the dispersal of their larvae[58]. However, the divergence of these clades may have occurred in the past during the Pleistocene lowering of sea level.

The average rate of nucleotide changes in the *Cyt b* gene observed in this study (1.8–2.2%) between various lineages lies in the range described for marine fishes (between the extremes of sharks and mammals (2.5% and 1.0% per million years respectively)). This indicates that the split between the lineages dates back to the mid-Pleistocene. In addition, the co-occurrence of the Haplogroup M of Rohfritsch and Borsa[40] and the Indian Ocean

population to form a major lineage and their heterogeneous geographical distribution suggests that differences may be due to vicariance. The average genetic divergence between the haplogroup M and the Indian Ocean population corroborates the above findings (while considering the whole population (2.2%) vs only these two (haplogroup M and IO population) (1.8%)).

Past geological and climatic events have undoubtedly played a major role in this inferred population expansion. The paleogeography of IMA changed dramatically during the Quaternary period[10]. During the last interglacial period, there were marine connections between the Indian Ocean and the south China Sea via the Strait of Singapore[85]. These connections may have allowed *D. russelli*, an essentially pelagic species to freely migrate and expand its population across this region at that time. Estimation of the time since expansion of various lineages of *D. russelli* suggests the timing of these events is consistent with the cyclic sea level rises that occurred during the late Pleistocene between 1,600,000 to 10,000 years ago[86]. The land bridges, which were later exposed as sea level fell, impeded the spread of *D. russelli*. Furthermore, the last glacial maximum (LGM), 30,000 to 19,000 years ago led to the final decline in sea level up to the present[86,87]. A substantial portion of Sunda and Sahul shelves was exposed as the sea level declined with glaciation up to 200 m below their present level[82]. This process would have also impeded the dispersal of many marine taxa. By this time the adjacent South China Sea considerably shrank in size and transitioned into a semi closed marginal sea exposing a massive low gradient on the Sundaland carton[86]. In addition, around this time the modern Malayan Penisula, Borneo and Sumatra formed highlands to the south[88] adding yet another potential barrier to population migration. The co-occurrence of an IMA lineage and an IO lineage (clade 2 and clade 3) as well as their distinct geographic distributions, as seen in this study, can be attributed to the remnants of these earlier marine links and the emergence of geographic barriers brought about by ice ages associated with the Pleistocene events respectively. Such isolated marine populations experienced extensive inbreeding leading to the accumulation of genetic variation and the structuring of their population at the genetic level. Similar scenarios have been reported for the genetic heterogeneity of many marine species between oceans[68,69]. MSN analysis also supported this structuring of *D. russelli* across the IMA and between the Indian Ocean and the IMA.

Marine species with high dispersal potential usually display high level of gene flow resulting in low level of genetic structuring[89]. This would limit or reduce adaptive divergence among local populations[90]. The low and non-significant $F_{ST}$ values observed here among the five *D. russelli* populations from Indian waters are consistent with the pattern of *Cyt b* homogeneity and high gene flow (Nm = 208.34). Various evolutionary processes including migration, mutation and drift may play a role in determining genetic differentiation between populations. Thus, a highly migratory species with a large population has a tendency to show low level of population differentiation in contrast to species with small populations and reduced migration rates[91]. Several other investigations have also reported relative panmixia in marine species across Indian waters. For example, an apparent lack of genetic structure was observed in Bigeye tuna (*Thunnus obesus*)[27] and *Euthynnus affinis*[67] based on the analysis of mitochondrial control region. Furthermore, mitochondrial (*ND2*) and nuclear DNA (microsatellites) analyzes of *Xiphias gladius* have shown its homogenous distribution throughout the Indian Ocean[68].

The present study also identified a significant population differentiation between the IMA and the Indian Ocean (Nm = 0.13). Although it is true that gene flow between populations can often prevent subpopulation isolation[92,93], this relationship is not as simple as it first appears. The hierarchical AMOVA results revealed significant $F_{ST}$ and $F_{CT}$ values indicative of the genetic structure among populations of *D. russelli*. This was confirmed by spatial genetic heterogeneity (k = 2 in SAMOVA) between groups comprising the populations in the IMA vs Indian Ocean. In addition, the geographical distance also contributed to the divergence among populations. Overall genetic distance increased with geographical distance consistent with isolation by distance. Thus, a more plausible explanation for the observed population differentiation of two distinct groups of *D. russelli* is the combined effects of several factors including historical physical isolation during the Pleistocene, larval dispersal factors, biotic and abiotic features of the ocean basins, ocean currents and geographical distance.

The movement of marine fishes, especially as larvae and adults, can greatly be influenced by Ocean surface currents. In the Indian Ocean, it is largely influenced by monsoon currents, also known as monsoon drifts, which flow between the Bay of Bengal and the Arabian Sea[94]. In contrast, the Kuroshio current, the largest surface current in the South China Sea affects the waters surrounding the IMA[95]. These two sea surface currents can contribute to, and also act as, powerful factors in genetic differentiation among *D. russelli* populations between the IMA and the Indian Ocean.

The significant genetic differentiation detected between Indian Ocean and IMA needs to be corroborated by advanced genomic markers like SNPs detected through genome scans. Genome scans also will provide insights regarding loci under selection in response to heterogeneous environmental conditions of the Indian ocean and IMA[96,97]. In addition, tagging programs and microchemical analyzes of specific structures (eg: otolith) may also be helpful to devise management measures for *D. russelli* in the Indian Ocean. Finally, a comprehensive understanding of reproductive strategies, population dynamics in various regions, and spawning areas within the Indian Ocean is required to fully understand the nature and behavior of its population[98].

## Conclusion

The results of this study demonstrated that the Indian Ocean *D. russelli* constituted a single panmictic population and can be managed as a single stock. This study revealed two discrete populations of *D. russelli*, Indian Ocean populations and IMA populations. The inferred demographic history indicates that *D. russelli* populations may have experienced a potential population expansion during the Pleistocene. The subsequent appearance of a barrier to gene flow may have limited their ability to migrate between the Indian Ocean and the IMA as seen in the extant populations.

## Materials and methods

### Sample collection

A total of 125 *D. russelli* individuals were collected from the east (Odisha (Puri) and Tamil Nadu (Chennai)) and west (Gujarat (Veraval), Mangalore (Karnataka) and Kerala (Cochin)) coasts of India between 2019–2020 (Fig. 4) A piece of caudal fin basal tissue was collected from each individual and preserved in 95% ethanol until DNA isolation. The fish sample used in this study was treated in accordance with the recommendations made by De Tolla et al.[99] for the handling and use of fish in research. The protocols were approved by the ethical committee of the ICAR—Central Marine Research Institute, Kochi. These methods are also reported following ARRIVE guidelines (http://arriveguidelines.org).

### DNA isolation and PCR amplification

Genomic DNA was isolated from tissue using the standard phenol–chloroform protocol[100]. The isolated DNA was then eluted in T.E. buffer (1 ×) and the DNA samples were stored at 4 °C prior to amplification by PCR. The quality and quantity (concentration) of the extracted DNA was checked using gel electrophoresis or NanoDrop One Spectrophotometer (Thermoscientific, USA). To facilitate comparative analysis, a new primer set was designed that targets a 933 bp fragment of mitochondrial *Cyt b* gene sequences spanning the regions analyzed by Rohfritsch and Borsa[40]. The primer sequences were as follows: 5′-AATCTCCGTAAAACCCACCC-3′ (forward) and 5′-AAAAACTAGGAATAGGAGGAAGT-3′ (reverse). The primers were designed based on the aligned full-length *Cyt b* gene sequences of carangids using Geneious R7 (Biomatters Ltd., New Zealand). The PCR reaction mixture consisted of 15.6 µl of sterile deionized water, 2 µl of 10 × buffer, 10 mM of each dNTP, 10 µM of each oligonucleotide, 1 unit of Taq DNA polymerase, and ~ 50 ng of genomic DNA as template. Amplification conditions were: initial denaturation at 94 °C (4 min); 32 cycles of 94 °C (30 s), 55.4 °C (30 s), 72 °C (1 min 10 s) and a final extension at 72 °C (10 min) before the reaction termination at 4 °C. Amplification of this 20 µl reaction volume was performed in a Biorad T100 thermal cycler (Bio-Rad, USA). Intron 1 of the aldolase b (*DrAldoB1*) gene was targeted with the following primers; 5′-GCTCCAGGAAAGGGAATCCTGGC-3′ (forward) and 5′-CTC GTGGAAGAAGATGATCCCGCC-3′ (reverse)[101]. PCR amplification was performed in a Biorad T100 thermal cycler (Bio-Rad, USA) with a final volume of 25 µl, consisting of 12.5 µl of 2 × EmeraldAmp® GT PCR Master Mix (TaKaRa, Shiga, Japan), 0.3 µl of each primer (10 mM each), 2.0 µl of genomic DNA (50 ng/ml) followed by nuclease-free water to achieve final reaction volume. Thermal cycling conditions were as follows: initial denaturation at 94 °C for 4 min, followed by 35 cycles of 30 s denaturation at 94 °C, 12 s annealing at 52 °C, 20 s extension at 72 °C followed by final extension of 3 min at 72 °C. DNA amplification products were then separated in 1.5%

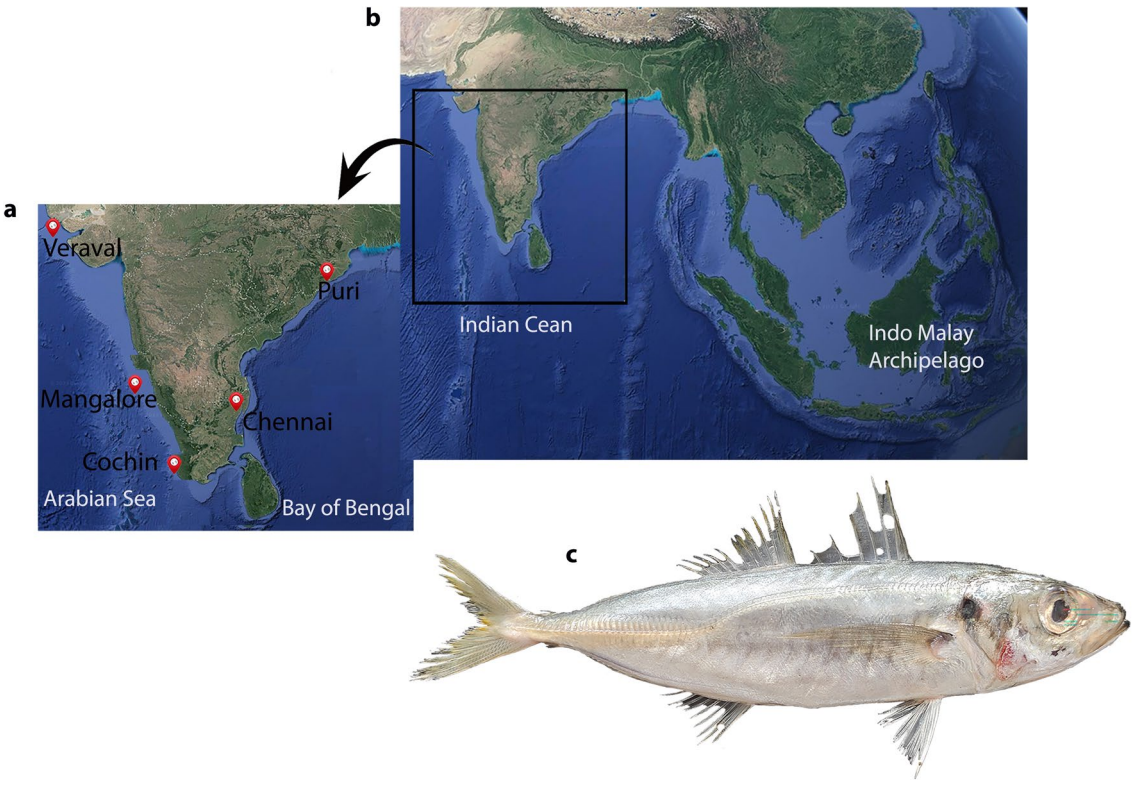


**Figure 4.** Map of sampling sites, site used for comparative analysis and targeted species. (**a**) Samples were collected from respective landing sites (red pin) from 5 localities of the Indian Ocean. (**b**) A comparative analysis of *D. russelli* populations in the Indian Ocean and the Indo-Malay Archipelago. (**c**) Specimen of Indian Scad, *D. russelli*. The map was obtained from Google Earth and the sampling sites were marked using the placemark tool in Google Earth available at http://earth.google.com. The images were then combined and edited manually in Adobe Photoshop CS6.0.

agarose gels at 90 V in 1× Tris–borate–EDTA (TBE) buffer, stained with ethidium bromide and visualized using a gel documentation system (Bio-Rad, USA). All PCR products were then sequenced using Sanger sequencing in both the forward and reverse directions. The nucleotide sequences were checked visually to ensure that the sequence information was consistent in both directions. The sequences were also verified by comparison with known sequences from GenBank.

## Data analysis

Both forward and reverse raw sequences were edited and assembled after removing the ambiguous bases in MEGA X[102]. The edited sequences of each locus were aligned with Clustal W implemented in the same software. The coding sequences of *Cyt b* were translated into amino acids, checked for stop codons and then back translated into the corresponding nucleotide sequences. A total of 933 bp sequences of *Cyt b* and 212 bp sequences of *DrAldoB1* were obtained for the subsequent analysis. Further, *Cyt b* (accession numbers: AF307494–AF307510 and AF515757–AF515759) and *DrAldoB1* (accession numbers: AF500103–AF500105) gene sequences deposited from IMA were also retrieved for comparative analysis. Prior to data analysis, the PHASE[103] program implemented in DnaSP6[104] was used to resolve heterogeneous sites in *DrAldoB1* sequences and to reconstruct haplotypes.

## Genetic diversity analysis

Genetic distance within and between populations was estimated using K2P model, selected based on the lowest Bayesian information criterion (BIC) score using MEGA X[102]. These values were also used to assess the possibility of occurrence of subspecies or cryptic species (if intra-specific variation exceeds the threshold of 2% in marine species)[105–108]. The complete aligned dataset was analyzed for nucleotide variable sites, parsimony informative sites, haplotype number, haplotype frequencies and nucleotide frequencies in DnaSP6[104]. The ratio of transition to transversion was analyzed using MEGAX[102].

## Population genetic structure analysis

Population pairwise $F_{ST}$ values were calculated using ARLEQUIN v3.5[109]. Hierarchical analysis of molecular variance (AMOVA) was executed to estimate population subdivision and structuring at different hierarchical levels using ARLEQUIN v3.5[109]. The AMOVA analysis was performed at three levels: among the Indian Ocean population (1), among the IMA population (2) and between the Indian Ocean and the IMA populations (3). For the Indian Ocean population, a hierarchical AMOVA analysis was performed by partitioning the variance among and within populations. (i) one gene pool comparison considering the whole population as a single gene pool, (ii) two gene pools [West coast (Cochin, Mangalore and Veraval); East coast (Chennai and Puri)] (iii) three gene pools [North West (Veraval); South West (Mangalore and Cochin); East (Chennai and Puri), and (iv) four gene pools [North West (Veraval); South West (Mangalore and Cochin); South East (Chennai) and North East (Puri)]. Further the variation was assessed among groups, within groups and within populations. The IMA population was divided into two geographically distinct gene pools based on the previous findings on their distribution[40]: Makassar Strait and Sulawesi Sea populations vs the populations from the entire western region of IMA. For the comparative analysis, the whole data set was divided into two groups: one comprising the populations from the Indian Ocean and the other comprising the entire population from the IMA. The variance among populations and the relative contribution of variance were estimated at three different levels; $F_{ST}$ (variation among individuals within populations), $F_{SC}$ (variation among populations within region) and $F_{CT}$ (variation among regions)[110]. In addition, the spatial structure was examined using spatial analysis of molecular variance (SAMOVA) v2.0[111,112] to identify groups of populations that were geographically homogenous and maximally differentiated from each other. Gene flow (Nm) between populations based on both haplotype and sequence statistics was estimated according to Nei[113] and Hudson et al.[114] using DnaSP6[104]. Genetic differentiation estimates based on $H_{ST}$, $N_{ST}$ and $K_{ST}^{*}$ were determined in DnaSP6[104]. The isolation by distance or Mantel test has been conducted to confirm the relationship between genetic distance and geographical distance[115].

## Phylogenetic analysis

The phylogenetic relationship among haplotypes was examined by constructing a phylogenetic tree based on Bayesian inference (BI) in MrBayes with 1,000,000 Markov Chain Monte Carlo (MCMC) generations. Posterior distributions were sampled for every 10,000 generations. The sequence of *D. macarellus* (NCBI, GenBank Accession No. NC_026718) was employed as an outgroup for phylogenetic tree construction based on *Cyt b* sequences and the sequences of *D. macarellus* (Accession No. AF500103) and *D. macrosoma* (Accession No. AF500104, AF500105) were used as outgroups for phylogenetic tree construction based on *DrAldoB1* sequences. A network diagram of all haplotypes was constructed based on median joining calculations as implemented in PopARTv1.7[116,117].

## Demographic history

The historical demographic expansion of each population was tested using Tajima's D[63] and Fu's F[61] statistics in ARELIQUIN v3.5[109]. In addition, Harpending's raggedness index, *Hri*[64] implemented in ARLEQUIN v3.5[109] and the mismatch distribution[118,119] implemented in DnaSp6[104] were applied to find out whether the sampled populations were demographically stable or expanding or decreasing over time. Both tests were applied in order to detect whether the populations deviate from what would be expected from a sudden population expansion model. A significant *Hri* value ($p < 0.05$) rejects a model of sudden population expansion[112].

The precise time for expansion was calculated using the equation $\tau = 2ut$[118]; where t is the expansion time. 2u was calculated using the equation $2u = \mu k$[120], where μ is the mutation rate which is 1–2%/site/year for the mitochondrial *Cyt b* gene in fishes (Carangidae)[121–123] and k is the number of nucleotides covered in the data (307 bp).

## Data availability

All data generated or analyzed during this study is included in this published article (and its supplementary information files). In addition, the sequence data generated in this study is available in the NCBI-GenBank under the accession numbers: OR188721 to OR188758 and OR546246 to OR546278 (https://www.ncbi.nlm.nih.gov/genbank/).

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## Author contributions

A.J. performed the experiments, analyzed and interpreted the data, and prepared the main manuscript text. S.S. conceptualized and coordinated the study and reviewed and edited the manuscript. S.K.R., A.A.P. and S.J.K. participated in the sample collection. N.R. and N.K. participated in the data analysis. A.G. supervised the study.

## Competing interests

The authors declare no competing interests.

## Additional information

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DAAD

# Complete mitochondrial genome of the Indian scad *Decapterus russelli*

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Mitochondria contain their own genome, a circular DNA molecule present in several copies per organelle. The mitochondrial (mt) genome is essential for life in almost all eukaryotes and repertoire of encoded genes is extremely conserved. Mitochondrial genes are popular genetic markers for evolutionary studies because they are effectively single copy, they show clonal pattern of inheritance and reduced effective population size. Generally, mtDNA is considered as a good molecular marker for phylogenetic analysis among fish taxa. However, the use of short mitochondrial gene fragments exhibit limitations in resolving complicated phylogenetic relationships in many fish lineages. The supplementary informative sites from longer DNA sequences (e.g., mitochondrial genomes) allow these deeper branches and higher level relationships to be more fully resolved.

An accurate phylogeny is essential for the complete understanding of vertebrate evolution. Therefore, questions of vertebrate relationships have been a primary focus in the field of molecular systematics. Fossils can provide evidence of morphological change, but the genetic changes associated with evolution can only be discerned from comparisons among extant taxa. Here arises the need for mitogenome characterization, by which one can delineate the relationship among extant taxa and thereby provide accurate results regarding evolutionary relationship at the genetic level. Genomic information is considered to be reliable for the efficient implementation of strategies to study evolutionary relationships, phylogeography and phylogeny.

Since Mitochondrial DNA (mtDNA) is an important model system in aquaculture research, the characterization of complete mitochondrial genome of fish species could contribute to the studies on its phylogenetics and provide strategies for fisheries management. The fishes of the family Carangidae are an important group of exploited pelagic resources of India. Carangids constitute the third most important resource, ranked next to the Indian oil sardine and Indian mackerel. The Indian scad, *Decapterus russelli* is one of the most exploited pelagic fish species in this family and is distributed on both east and west coasts of India. The complete mitogenome of this species was determined by overlapped Polymerase Chain Reaction. The circular mitochondrial DNA molecule is 16542 base pair (bp) in length which included a standard set of 13 protein coding genes, 22 transfer RNA genes (tRNA) genes, 2 ribosomal RNA genes (12S rRNA and 16S rRNA), and 2 non coding regions; control region and origin of light strand replication. Most of the genes were encoded on the heavy strand except ND6 gene and 8 tRNA genes (Ala, Asn, Cys, Gln, Glu, Pro, Ser (UCN) and Try), which were encoded on the light strand. The total length of the protein coding genes was 11425 bp accounting for 69% of the entire genome. The organization of the genome is similar to other typical vertebrate mitochondrial genomes. The overall base composition of the genome is 27.5% A, 30.2% C, 25.4% T and 16.9% G showing an obvious anti G bias observed in teleosts. These sequence data could provide useful information for further studies in fishery science such as studies on molecular phylogenetics and conservation genetics.

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## Structure and variation of Carangidae mitochondrial control region

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The vertebrate mitochondrial genome, which plays a crucial role in cell metabolism and organism function, is ~15-20 kb in size and consists of 13 protein-coding genes (PCGs), 22 tRNA genes, 2 rRNA genes and a non-coding region (control region). The PCGs, tRNA and rRNA have a relatively low mutation rate because they are subject to strong purifying selection. In contrast, the control region evolves at a high rate compared to the other parts of the mitogenome, and these high rates of sequence divergence are mostly localized in the variable flanking region, which is adjacent to the conserved sequence motifs. Comparative mitogenomics of 37 carangids revealed high variability in the mitochondrial control region due to length variations and the accumulation of base substitutions, insertions and deletions. We have detected the 3 central conserved sequence blocks (CSB- F, -E, -D) and 3 conserved sequence blocks (CSB- 1, -2, -3) in all taxa. Conserved sequence blocks (CSB-1, -2, and -3) exhibit a higher level of divergence in carangids with interspecies substitutions, insertions, and deletions. The CSB- D was highly conserved among carangids and exhibited high sequence similarity between species and CSB-F and –E showed sequence variation. Some species lacked a 'GTGGG'- box which is a typical feature of CSB- E in teleosts. These variations imply rapid evolution of the primary structure in the Carangidae control region, which can provide information for understanding their structural and functional relationship. Furthermore, these conserved sequence motifs formed similar secondary structures in Carangidae although their primary sequences are not conserved. Therefore, the data reinforce that although there is sequence divergence in the conserved sequence motif, their structural elements and locations appeared to be conserved in Carangidae and some selective constraints on these regions acted to maintain their structure and function.

**Keywords:** Carangidae, Control Region, Central Conserved Sequence Blocks, Conserved Sequence Blocks

# 3.4 Population Genetic Structure of Indian Scad, *Decapterus russelli* along Indian Coast Inferred from Nuclear *aldolase b* Gene Sequences

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**Keywords:** Population genetics, Indian ocean, Pelagic fish, Carangidae, Nuclear DNA

## Introduction

The Indian Scad, *Decapterus russelli* is a benthopelagic marine shoaling fish found at depths ranging from 40 to 300 metres. The species' distribution was traced to depths of 200 m along India's west coast and 300 m along east coast. It contributes a significant portion of India's pelagic resources, ranking next to Indian oil sardine. Previous research on the geographical structure of this species using truss morphometric analysis along the Indian coast (Sen et al 2011) demonstrated that the species is composed of spatially structured populations that should be managed independently. However, no attempts have been made to investigate the genetic basis of this structuring. Thus, studying the geographical structure of its population at the genetic level is critical for making effective management recommendations.

## Materials and Methods

A total of 125 specimens of *D. russelli* were collected from 5 localities along the India coast. The total DNA was extracted from the muscle tissue using the standard phenol chloroform method (Sambrook 2001). PCR amplifications were carried out in a final volume of 20µl containing 50ng of genomic DNA, 10mM of each dNTP, 10µM of each primer and the buffer recommended by the polymerase suppliers. PCR cycling consisted 4 min at 94°C, 32 cycles of 30 s at 94°C, 12 s at 52 °C, 20 s at 72 °C followed by 3 min at 72°C. The products were then sequenced completely in both directions by performing Sanger sequencing. 310 bp sequence of aldolase b obtained were aligned using clustalW in MEGA X. The genetic diversity indexes including number of alleles (h), haplotype diversity (Hd) and nucleotide diversity ($\pi$) were calculated using DnaSPv5.0. Genetic distance was calculated with MEGA X. Pairwise $F_{ST}$ values were estimated also with DnaSPv5.0. In order to test for molecular signatures of demographic expansion pairwise mismatch distribution analyses as well as Tajima's D and Fu's FS were performed inDnaSPv5.0.

## Results and Discussion

A 210 bp sequence of intron one of *Aldolase b* was successfully sequenced from 125 individuals (250 alleles). There were 54 unique alleles identified.The overall haplotype diversity was high (0.9075) whereas the global nucleotide diversity value was low (0.02303). We identified 131 loci with only one allele. As observed no obvious population structure was detected either related to the coast or related to the geographical distance. The minimum spanning network showed a star shaped pattern. One haplotype was common for all the geographical location. The common haplotypes within the network are inferred to be ancestral, whereas the tip haplotypes are derived or descended from ancestral (internal) haplotypes. Thus, the occurrence of star like pattern radiating from these major haplotypes suggest that *D. russelli* population have undergone recent demographic expansion.

Genetic differentiation among the population from different location was tested using $F_{ST}$ pairwise comparison and most of them were significant. Genetic variation within population was high at 94.71% revealing most of the variation was present within populations. A non-hierarchical AMOVA analysis showed significant global $F_{ST}$($F_{ST}$ = 0.04655, P<0.05). The estimated $F_{SC}$ and $F_{CT}$ value in the hierarchical AMOVA based on the two groups failed to reveal statistically significant values (p>0.05). The Mantel test revealed lack of significant correlation between genetic differentiation ($F_{ST}$/ (1-$F_{ST}$)) and geographical distance among tested population, hence no evidence of isolation by distance. The Tajimas D and Fu's Fs values observed for all populations were negative except puri which showed a positive Fu's Fs. It indicates these populations experienced a selective sweep or recent population expansion. In addition, the Harpending's raggedness index, *Hri*= 0.01854, SSD = 0.0118 (p=0.2) supported the recent population growth hypothesis. Mismatch distribution of combined population showed unimodal pattern indicating a sudden expansion in population.



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Sen S, Jahageerdar S, Jaiswar A K, Chakraborty S K, Sajina A M and Dash G R 2011. Stock structure analysis of *Decapterus russelli* (Ruppell, 1830) from east and west coast of India using truss network analysis. *Fisheries Research* **112**(1-2): 38-43.

*Corresponding author's email: anjalijose2@gmail.com

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**AG-O1**

# COMPARATIVE PHYLOGENETICS OF THE FAMILY CARANGIDAE WITH SPECIAL REFERNCE TO THE INDIAN SCAD, *DECAPTERUS RUSSELLI*.

**Anjaly Jose[1,2*], Sandhya Sukumaran[1], Neenu Raj[1]**

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Mitochondrial DNA is generally considered a good molecular marker for phylogenetic analysis among fish taxa. However, short mitochondrial gene fragments exhibit limitations in resolving complicated phylogenetic relationships in many fish lineages.The additional informative sites from longer DNA sequences (mitochondrial genomes) allow these deeper branches and higher-level relationships to be more fully resolved. To delineate the phylogenetic relationship of *D. russelli* among carangidae, we characterized its complete mitochondrial genome and the available mitogenome sequences of all other carangid species were obtained from Gen Bank. The complete mitogenome sequence of *L. crocea* was incorporated as an out-group. Multiple alignments for the 13 concatenated protein coding sequence from *D. russelli* and 36 other carangidae species were generated for the subsequent phylogenetic analysis. Phylogenetic relationship was inferred by constructing tree based on Bayesian inference method. The best model, GTR+G+I was selected based on Akaike Information Criterion by using the maximum likelihood statistical method in MEGA X. Phylogenetic analysis was performed using Mr Bayes. The final phylogenetic tree was displayed, annotated and embellished in Fig Tree. The phylogenetic position of *D. russelli* is shown relative to 36 species of carangids based on 1380 bp sequences. The result implied that 37 species formed 3 lineages corresponding to the subfamilies Caranginae, Naucratinae, and Trachinotinae. The reconstructed phylogenetic tree places *D. russelli*in the same branch with other *Decapterus*spp. and forms a sister group to the genus *Trachurus.* The findings will help future research on molecular taxonomy, population genetics, evolution and molecular phylogeny of *D. russelli* and its relatives.

**Keywords:** Phylogeny, Carangidae, Decapterus, mitogenome

## BABO1

# POPULATION GENETIC STRUCTURE OF THE INDIAN SCAD, *Decapterus russelli* ACROSS INDIAN OCEAN AND INDO-MALAY ARCHIPELAGO

**Anjaly Jose[12*], Sandhya Sukumaran[1] Neenu Raj[1] and Nisha K[1]**

[1]*Marine Biotechnology Fish Nutrition and Health Division, ICAR-Central Marine Fisheries Research Institute, Ernakulam North P O, Kochi, Kerala, India-682018*

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The Indian scad, *Decapterus russelli* is an important pelagic Carangidae widely distributed throughout the Indian Ocean and Indo-West Pacific. Although widespread in the Indian Ocean, information on genetic structure of this species is lacking compared to its Indo-Malay Archipelago (IMA) counterparts. The present study was conducted to investigate the genetic population structure of *D. russelli* using mitochondrial (*Cyt b*) and nuclear (*DrAldoB1*) markers along Indian waters. The results indicated the occurrence of a single panmictic stock throughout the Indian Ocean region. Phylogenetic analysis revealed that there is no geographical clustering of lineages. However, significant genetic differentiation has been observed between the Indian Ocean and IMA populations. Pairwise FST comparisons, AMOVA and SAMOVA revealed the existence of two distinct genetic stocks of *D. russelli* in the Indian Ocean and the IMA. The observed genetic variation between populations was high. A plausible explanation for the observed genetic differentiation between the Indo-Malay Archipelago and the Indian Ocean regions suggests the influence of historical isolation, sea surface currents, and biotic and abiotic characteristics of the ocean. Also, there was a significant association between genetic distance and geographic distance in a manner consistent with isolation by distance. This led to the development of a phylogeographic break of this species between these regions. The results of these findings suggest that Indian Ocean *D. russelli* should be managed as a single stock throughout its range. In addition, the Indian Ocean stocks and the Indo-Malaysia stocks can be managed separately.

**Keywords:** Carangidae, Indian scad, population structure, molecular markers, Indian Ocean, Indo-Malay Archipelago

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No. MU/EXB/Ph.D./CR.25/Biosc/2022-23/E.25

Office of the
Registrar (Evaluation),
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Date: 15.06.2022

## OFFICIAL MEMORANDUM

Sub: Registration for Ph.D. Degree Programme in **Biosciences.**

Ref: 1) Regulation Governing the Degree of Ph.D. Notification No. MU/ACC/CR.9/2009-10/A2, Dated 03.09.2010 and Circular No. MU/ACC/CR.49/2015-16/A2 dated 21.05.2016 relating to guidelines for implementing the regulation.
2) Provisional admission O.M. No.MU/ACC/Ph.D./CR.62/2018-19/A3, Dated 21.12.2019 of the Registrar, Mangalore University.
3) Letter No.MU/ACC/Ph.D-Reg.62/2018-19/A3, Dated: 11.01.2022 of the Registrar, Mangalore University.

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The application of **Ms. Anjaly Jose., Researcher in Biosciences, Central Marine Fisheries Research Institute (CMFRI), Ernakulam North P.O, Cochin, Kerala - 682018** has been accepted for registration for Ph.D. programme of this University with effect from **20.08.2019.** She is permitted to work and prepare a thesis in **"Biosciences"**, on the topic entitled **"INVESTIGATIONS ON POPULATION STRUCTURE AND INTRA-SPECIFIC GENETIC DIVERSITY OF INDIAN SCAD, *DECAPTERUS RUSSELLI* (RUPPELL, 1830) FROM INDIAN WATERS"** under the guidance of **Dr. Sandhya Sukumaran, Senior Scientist, Central Marine Fisheries Research Institute (CMFRI), Ernakulam North P.O, Cochin, Kerala - 682018** subject to the following conditions:

1. The researcher shall undertake the research work on **FULL– TIME** basis. She shall work in the **Central Marine Fisheries Research Institute (CMFRI), Ernakulam North P.O, Cochin, Kerala - 682018.**
2. The researcher is not permitted to join or register for any other degree programme of this University or any other University during the period of registration.
3. The title of the thesis can be changed or modified/altered with the prior approval of the competent authority of Mangalore University.
4. The researcher shall pay annual / term fee as fixed by the University from time to time along with half yearly progress report.
5. a) The researcher shall submit half yearly progress report regularly to the Registrar (Evaluation) through the guide and Chairperson of the Department/Head of the Institution during June and December.

   b) Progress report may contain new data generated or techniques/concepts developed /standardization in research methodology/discussion of the work done etc.
6. If a researcher fails to submit two consecutive half yearly progress reports or the progress reports submitted by the researcher are found to be unsatisfactory by the Doctoral Committee, the University reserves the right to cancel the registration.

.....2

07 The full-time researcher may submit the thesis only after a period of three years from the date of registration. The maximum period for submission of thesis is five years from the date of registration. Extension beyond the above period up to two years may be permitted by the University on the recommendation of the research guide along with payment of the prescribed fee. The application for extension should be submitted well-in-advance before the expiry of the said period. If he/she fails to submit thesis even after extension, he/she shall re-register and submit the thesis within two years.

08 The researcher may be allowed to convert the registration from full-time to part-time and vice-versa on valid grounds. In such cases the period for submission of thesis may vary.

09 Prior to submission of thesis, the researcher shall participate in a pre-submission colloquium by submitting an application along with six copies of synopsis of the research work carried out to the Registrar (Evaluation) through the research guide and Chairperson of the Department/Head of the Institution and shall present the details of research work in the pre-submission colloquium. He/she shall have published a minimum of two research papers(published or accepted in reputed journals)/presented a minimum of two research papers at the National Level Seminars or hold patents (accepted or granted) on the basis of his/her research before being eligible for participating in the colloquium.

10 After obtaining approval of the Doctoral Committee to submit thesis, the researcher shall submit 12 copies of the final synopsis of the thesis along with a soft copy to the Registrar (Evaluation) through the concerned guide and Head of the Department or institution/College. Within three months after the submission of final synopsis, the researcher shall submit four copies of the thesis and a soft copy along with the application and prescribed fee paid challan to the Registrar (Evaluation) through the concerned guide and Head of the Department or Institution/College. The extension upto three months may be granted on payment of penal fee prescribed by the University. Further additional time may be granted on payment of penal fee. In all cases the total period shall not exceed maximum period allowed for submission of thesis.

11 The thesis shall be accompanied by a certificate from the guide that the thesis submitted is a record of the research work carried out by the researcher during the period of the study, and that it has not previously formed the basis for the award of any Degree/ Diploma/Associateship/Fellowship or other similar titles to any other researcher in any other University/Institution. `NO DUE CERTIFICATES' from the Chairperson of the Department / Head of the Institution and the University Library and Hostel Warden (if the researcher is staying in the University Hostel).

16/6/2021

**REGISTRAR (EVALUATION)**

To:

Ms. Anjaly Jose
Researcher in Biosciences,
Central Marine Fisheries Research Institute (CMFRI),
Ernakulam North P.O, Cochin, Kerala - 682018.

*Copy to :*

1. The Registrar, Mangalore University, Mangalagangothri - for information.
2. The Chairman, B.O.S/D.O.S. in Biosciences, Mangalore University, Mangalagangotri,
3. The Director, Central Marine Fisheries Research Institute (CMFRI), Ernakulam North P.O, Cochin, Kerala - 682018.
4. Dr. Sandhya Sukumaran, Senior Scientist, Central Marine Fisheries Research Institute (CMFRI), Ernakulam North P.O, Cochin, Kerala – 682018.

# MANGALORE UNIVERSITY

No. MU/EXB/Ph.D./CR.25/Biosc./2022-23/E25

Office of the
Registrar(Evaluation)
Mangalagangotri - 574199
Karnataka State, India.
Dated: 04.11.2024

## OFFICIAL MEMORANDUM

Sub.: Permission to Ms.Anjaly Jose, to submit Ph.D. thesis. - reg.

Ref.: 1) The Regulation governing Doctoral degree programme vide Notification No.MU/ACC/CR9/2009-10/A2, Dated 03.09.2010 and guidelines circular dated 21.05.2016.
2) Her synopsis submission letter dated 25.04.2024.
3) Proceedings of the Doctoral Committee meeting held on 18.10.2024.

---

With reference to the above, Ms. Anjaly Jose, Researcher, Dept. of Biosciences, ICAR-Central Marine Fisheries Research Institute, Ernakulam, Kochi is permitted to submit the Ph.D. thesis within stipulated period, incorporating all the following suggestions recommended by the Doctoral Committee during pre-thesis submission colloquium.

1. Clearly outline the experimental design.
2. Ensure that the methodology is well-structured, detailed and replicable.
3. Make sure statistical results are properly interpreted and explained. Include p-values to indicate significant variation among the groups.
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7. Ensure coherence and flow in the writing to enhance readability.
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9. Incorporate a section discussing the potential societal contributions of the research findings. Provide a broader context for the work and emphasize its significance.

{Draft approved by R(E)}

Yashod 4/11/2024

For REGISTRAR (EVALUATION)

To: The concerned.

***Copy to :***